



## **Transport and survival of *Cryptosporidium parvum* oocysts in soil columns following applications of raw and separated liquid slurry**

**Petersen, Heidi H.; Enemark, Heidi; Olsen, Annette; Mostofa Amin, M. G.; Dalsgaard, Anders**

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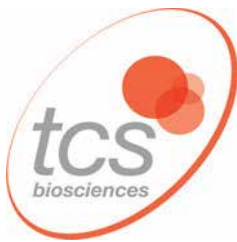
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# Scientific Programme

## TUESDAY 27<sup>th</sup> MAY

**13.00-18.00**

**REGISTRATION**

Uppsala Konsert & Kongress, conference venue

**16.00-18.00**

**MINI-SYMPOSIUM: Childhood diarrhea - a global problem**

Chair: *André Buret, University of Calgary, Canada*

O1 "The Global Enteric Multicenter Study (GEMS) of Diarrheal Disease in Infants and Young children in Developing Countries".

*Karen Kotloff, University of Maryland*

O2 "The interaction of enteric infections and malnutrition: consequences for child health and development".

*Bill Petri, University of Virginia*

O3 "Cryptosporidium Strategy Development at the Enteric and Diarrheal Diseases Program, Bill & Melinda Gates Foundation"

*Ibrahim Khalil, Senior programme officer, Enteric Diseases, Bill and Melinda Gates Foundation*

Discussion

**18.00-18.15**

**OPENING CEREMONY**

**18.30-21.00**

**WELCOME RECEPTION**

SciLifeLab Uppsala, BMC

# Scientific Programme

**WEDNESDAY 28<sup>th</sup> MAY**

## **FOOD AND WATER TRANSMISSION**

*Chair: Kristin Elwin (UK)*

**8.30-9.00**

INVITED SPEAKER

O4 Foodborne and waterborne transmission of *Cryptosporidium* and *Giardia*

*Lucy Robertson (Norway)*

**9.00-11.00**

ORAL PRESENTATIONS

O5 Essentials of environmental monitoring for *Cryptosporidium* and *Giardia*.

Jerry Ongerth

O6 A national strategy for human *Cryptosporidium* infection.

Charlotte Axén, Ann-Christine Ring, Maria Egervärn, Torbjörn Lindberg, Linda Trönnberg, Jessica Beser, Anette Hansen, Martin Holmberg, Bo Svenungsson, Kaisa Sörén, Anna Nordström, Karin Troell, Jakob Ottoson and Annica Wallén Norell

O7  $\mu$ AQUA – a universal microarray for the detection of waterborne pathogens.

Bas Boots, Ciara Ní Chualáin and Nick Holden

O8 Selection of DNA aptamers for the detection of *Cryptosporidium parvum* in foods.

Brent Dixon, Asma Iqbal, Darija Muharemagic, Maxim Berezovski and Syed Sattar

O9 Wastewater treatment with *Moringa oleifera* seed extract: impact on turbidity and sedimentation of *Cryptosporidium parvum* oocysts.

Heidi Petersen, Ian Woolsey, Anders Dalsgaard, Heidi Enemark and Annette Olsen

O10 Investigation into a *Cryptosporidium* outbreak linked to a swimming pool.

Elaine Moriarty, Debbie Smith, Anthony Pita, Nigel French and Ian Shaw

O11 Swimming pool outbreaks of *Cryptosporidium* across England 1988 to 2012

Gordon Nichols, Rachel Chalmers and Adedoyin Awofisayo

O12 An outbreak of *Cryptosporidium parvum* across England & Scotland associated with consumption of fresh pre-cut salad leaves.

Gordon Nichols, Caoimhe McKerr, Goutam Adak, Russell Gorton, Rachel Chalmers, George Kafatos, Paul Cosford, Charlett Andre, Kevin Pollock and Stephen Morton

**11.00-11.30**

**COFFEE BREAK (FIKA)**

**11.30-12.45**

**INFECTION IN ANIMALS AND ZOOLOGICAL ASPECTS**

*Chair: Heidi Enemark (Denmark)*

ORAL PRESENTATIONS

O13 *Cryptosporidium* infection and associated calf-level risk factors in French dairy calves.

Carine Paraud, Christophe Chartier, Marie-Christine Dupuy, Mathieu Dumoulin, Isabelle Pors and Arnaud Delafosse

O14 *Cryptosporidium* infection in beef calves in Sweden.

Camilla Björkman, Lina Lindström, Carolina Oweson, Karin Troell and Charlotte Axén

O15 Detection and molecular epidemiology of *Giardia duodenalis* and *Cryptosporidium* spp. in asymptomatic adult cattle in Northern Spain.

David Carmona, Guillermo A Cardona, Begoña Bailo, José M Saugar, Carlos Marín and Isabel Fuentes

O16 The role of adult cattle in the transmission and persistence of *Cryptosporidium*.

Sarah Thomson, Beth Wells, Nicholas Jonsson, Elisabeth A Innes and Frank Katzer

O17 Transmission dynamics of *Cryptosporidium parvum* between beef farms in two regions of Scotland.

Emily J Hotchkiss, Sarah Thomson, Frank Katzer, Nicholas Jonsson and Elisabeth Innes

O18 Age related susceptibility of pigs to *Cryptosporidium scrofarum* infection.

Martin Kvac, Karel Nemejc, Michaela Kestranova, Dana Kvetonova, Pavla Wagnerova, Michaela Kotkova, Michael Rost, Eva Samkova, John McEvoy, and Bohumil Sak

**12.45-13.45**

**LUNCH**

**13.45-14.45**

## **INFECTION IN ANIMALS AND ZONOTIC ASPECTS**

*Chair: Camilla Björkman (Sweden)*

### **ORAL PRESENTATIONS**

O19 *Giardia* and *Cryptosporidium* in coyotes and wolves in northwestern Canada.

Emily J Jenkins, Michael Pawlik, Mandeep Bal, Brent Dixon, Brent Wagner, Karen Gesy, Janna Schurer, Brett Elkin and Helen Schwantje

O20 Occurrence of *Giardia* and *Cryptosporidium* in captive chimpanzees (*Pan troglodytes*), mandrills (*Mandrillus sphinx*) and wild Zanzibar red colobus monkeys (*Procolobus kirkii*).

John J Debenham, Fred Midtgaard, Rebeca Atencia, Benezeth M Mutayoba and Lucy Robertson

O21 Molecular characterization and genotyping of *Cryptosporidium* spp. in chicken and turkeys in Germany.

Yosra A Helmy, Jürgen Krücken, Georg Von Samson-Himmelstjerna, Karl-H Zessin and Hafez M Hafez

O22 Characterisation of *Cryptosporidium* in fish.

Una Ryan, Andrea Paparini, Mikayla Morine, Melanie Koinari, Kaising Tong, Susan Keuh and Alan Lymbery

O23 Identification of *Cryptosporidium* species in fish from the Lemman Lake in France.

Gabriela Certad, Jean Dupouy-Camet, Nausicaa Gantois, Ourida Hamouma-Ghelboun, Muriel Pottier, Karine Guyot, Sadia Benamrouz, Marwan Osman, Baptiste Delaire, Colette Creusy, Eric Viscogliosi, Eduardo Dei-Cas, Cecile-Marie Aliouat-Denis and Jérôme Follet

**14.50-15.45**

## **DIAGNOSTIC AND TREATMENT**

*Chair: Kurt Hanevik (Norway)*

### ORAL PRESENTATIONS

O24 Implications of molecular detection on surveillance of intestinal parasites.

Titia Kortbeek and Theo Mank

O25 Introduction of a *Giardia* typing service in Wales UK).

Kristin Elwin, Hannah Evans, Daniel Rh Thomas, Laia Fina, Sarah Jones and Rachel M Chalmers

O26 A puzzling relationship between albendazole metabolization and albendazole resistance in *Giardia duodenalis*.

Raúl Argüello-García, Maricela Cruz-Soto, Rodrigo Martínez-Espinoza, María L Bazán-Tejeda and Guadalupe Ortega-Pierres

O27 GiTox: a simple colormetric method for screening drug susceptibility in *Giardia duodenalis*.

Samantha J Emery, Jennifer H Gill and Ernest Lacey

**15.45-16.15**

## **COFFEE BREAK (FIKA)**

**16.15-17.20**

### ORAL PRESENTATIONS, CONT.

O28 Elaboration of a vaccine to immunize pregnant cows to protect newborn calves from *Cryptosporidium parvum* infection.

Karine Sonzogni-Desautels, Axel Renteria Flores, Momar Ndao, and Timothy Geary

O29 Efficacy of chitosan, a natural polysaccharide, against *Cryptosporidium parvum* development in infected hct-8 and caco-2 enterocytic cells.

Karim Adjou, Oleg Chihai, Alexandra Emmanuel, Veronique Laine-Prade, Aurelie Grasset-Chevillot, Jean-Philippe Marden, Eric Auclair and Isabelle Vallee

O30 External quality assessment program for *Cryptosporidium* spp. diagnosis: experience of the French ANOFEL *Cryptosporidium* national network.

Loic Favennec, Nathalie Kapel, Meja Radbodonirina, Jean Menotti, Claudine Sarfati, Claudine Pinel, Gilles Gargala, Francis Derouin, and French *Cryptosporidium* network

O31 Evaluation of *Cryptosporidium parvum* infectivity by Electrochemical Impedance Spectroscopy.

Jérôme Follet, Alfred DibaoDina, Alexis Vlandas and Vincent Senez

O32 A novel method for simple and sensitive detection of *Cryptosporidium parvum* by surfactant extraction treatment (SET) and quantitative reverse transcription-PCR.

Takahiro Sekikawa

**17.30-19.30**

## **WORKSHOP: Waterborne *Cryptosporidium* and *Giardia*: Regulatory and Legal Issues**

(See full programme on page 21)



**THURSDAY 29<sup>th</sup> MAY**

## **HOST-PARASITE INTERACTIONS AND PATHOLOGY - I**

*Chair: Carmen Faso (Switzerland)*

**9.00-9.40**

INVITED SPEAKER

O33 Host-parasite interactions in giardiasis: from effects on microbiota to immunomodulation.

*Andre Buret (Canada)*

**9.40-10.20**

ORAL PRESENTATIONS

O34 Determinants of *Cryptosporidium* and *Giardia* virulence. (20 minutes)

Kevin M Tyler, Maha Bouzid, Johanna Nader, Suha Al Naimi, Dong Xia, Audrey Dubourg, Kristin Elwin, Jonathan Wastling, Rachel Chalmers and Paul R Hunter

O35 Representational difference analysis (RDA) identifies up-regulated genes in the murine intestinal epithelial cell line (IEC-6) in response to *Giardia duodenalis* infection.

Showgy Y Ma'ayeh, Phillip T Brook-Carter, Mark R Sandeman

**10.20-11.25**

POSTER SESSION AND FIKA (COFFEE)

**11.25-12.30**

ORAL PRESENTATIONS, CONT.

O36 Analysis of the damage in epithelial cells exposed to surface proteins, metabolic and proteolytic enzymes secreted by *Giardia duodenalis* trophozoites.

Guadalupe Ortega-Pierres, Rocío Fonseca-Linán, Misael Gómez-Mondragón, Ariana Cabrera-Licona, Elisa Barroeta-Echegaray, Rosa M Bermúdez-Cruz, Silvia Espinosa-Matías, David Flores-Benítez, Arturo Raya-Sandino, Noemi Meráz-Cruz, José Luis Ventura, Alejandro Zentella-Dehesa, Staffan Svärd, Raúl Argüello-García and Lorenza González-Mariscal

# Scientific Programme

O37 Measuring the effects of *Giardia duodenalis* on transcellular and paracellular transport in enterocytes.

Kristoffer R Tysnes and Lucy J Robertson

O38 Characterization of *Giardia duodenalis* enolase and its role in the interaction with epithelial cells.

Elisa Barroeta-Echegaray, Rocio Fonseca-Liñan, Luisa M Bazán-Tejeda, Rosa M Bermudez-Cruz and Guadalupe Ortega-Pierres

O39 Metabolic shift from ceramide to sphingomyelin: Is it critical for cyst viability and excystation of *Giardia*?

Siddhartha Das, Trevor T Duarte, Tavis L Mendez, Atasi De Chatterjee, Sukla Roychowdhury, Igor C Almeida

O40 Interaction between *Cryptosporidium parvum* and the intestinal microbiota. Giovanni Widmer and Refaat Ras

**12.30-13.30**

LUNCH

**13.30-15.00**

**HOST-PARASITE INTERACTIONS AND PATHOLOGY - II**

*Chair: Guadalupe Ortega-Pierres (Mexico)*

ORAL PRESENTATIONS

O41 Activation of pathological immunity in giardiasis.

Steven M Singer, Aleksander Keselman, Jenny Maloney, Erqiu Li and Jennifer Marvin

O42 PPAR $\alpha$  and IL-17A responses associated with the intestinal immune response against the protozoan parasite *Giardia muris*.

Leentje Dreesen, Karolien Debosscher, Bart Staels, Erik Lubberts, Ed Claerebout and Peter Geldhof

O43 Flow cytometric method for characterization of human T cell responses against *Giardia lamblia*.

Christina Skår Saghaug, Dimitra Peirasmaki, Steinar Sørnes, Staffan Svärd, Nina Langeland, Kurt Hanevik

O44 Cryptosporidiosis induces caspase-1 driven cytokine cascade.

Jan R Mead, Brahmchetna Bedi and Nina N McNair

O45 Can infection with one genotype of *C. parvum* protect against infection with another?

Sarah Thomson, Beth Wells, Paul Bartley, Emily Hotchkiss, Maria Parigi, Marieke Opsteegh, German Canton, Alison Burrells, Jackie Thomson, Joao Luis Garcia, Nicholas Jonsson, Elisabeth A Innes and Frank Katzer

O46 DNA damage repair in *Giardia duodenalis*: characterization of putative rad52 gene.

Rosa M Martínez-Miguel, Diego Armando Martinez-Reyes, Xu Yuanyuan, Patrick Sung and Rosa M Bermudez-Cruz

O47 Electron microscopic study of in vitro axenic development of *Cryptosporidium parvum*.

Hebatalla M Aldeyarbi and Panagiotis Karanis

**15.00-15.30**

**FIKA AND POSTERS**

**15.30-17.20**

**MOLECULAR EPIDEMIOLOGY AND TYPING**

*Chair: Michelle Power (Australia)*

**ORAL PRESENTATIONS**

O48 Epidemiology and molecular genotyping of *Cryptosporidium* and *Giardia* in cattle, buffalo and humans in the Ismailia province of Egypt.

Yosra A Helmy, Jürgen Krücken, Christian Klotz, Karsten Nöckler, Toni Aebischer, Georg Von Samson-Himmelstjerna and Karl-H Zessin

# Scientific Programme

O49 Molecular typing of Swedish *Cryptosporidium* isolates during 2013 – few domestic cases caused by anthroponotic species/subtypes.

Marianne Lebbad, Jessica Beser, Jadwiga Winięcka-Krusnell, Anette Hansen, and Margareta Löfdahl

O50 *Cryptosporidium* increase in the summer of 2012 in the Netherlands analysed by GP60 genotyping.

Jeroen H Roelfsema, Simone M Cacciò, Hein Sprong, Joke WB Van der Giessen, Barbara Schimmer, Wilfrid Van Pelt and Titia M Kortbeek

O51 Comparative genomics of *Cryptosporidium hominis*: genetic recombination as a driving force for emergence of virulent subtypes. (20 minutes)

Lihua Xiao, Kevin Tang, Yaqiong Guo, Na Li, Michael Frace, Scott Sammons, Kristinen Knipe, Dawn Roellig and Yaoyu Feng

O52 Comparison of Sanger and Next Generation sequencing for typing *Cryptosporidium* isolates.

Una Ryan, Andrea Paparini, Rongchang Yang, Alexander Gofton, Mike Bunce and Josephine Ng-Hublin

O53 CryptoTyper, a software package for automated GP60 subtyping of *Cryptosporidium*.

Erik Alm, Jessica Beser and Marianne Lebbad

O54 Harmonisation of multi-locus subtyping schemes for public health investigations of *Cryptosporidium parvum* and *Cryptosporidium hominis*

Rachel M Chalmers, Stephen J Hadfield, Claire Alexander and Emily Hotchkiss

O55 Generation of a *Giardia duodenalis* biobank for functional epidemiology

Christian Klotz, Juliane Hahn, Ralf Ignatius, Petra Gosten-Heinrich and Toni Aebischer

O56 What is a *Cryptosporidium* isolate?

Alex Grinberg

17.30-19.00

## CLINICAL AND PUBLIC HEALTH

*Chair: Titia Kortbeek (Netherlands)*

### ORAL PRESENTATIONS

O57 Sequelae of human Cryptosporidiosis in patients living in Östersund and Skellefteå during two large waterborne outbreaks.

Johan Lindh, Micael Widerstrom, Michael Lilja and Stephan Stenmark

O58 Investigation of the long-term health sequelae following acute infection with *Cryptosporidium parvum*: an outbreak follow-up study.

Rhianwen E Stiff, Angharad P Davies, Brendan W Mason, Rachel Chalmers

O59 Treatment with cytokine modulating agents - an emerging clinical risk factor for cryptosporidiosis.

Angharad P Davies, Andrew Deacon, Uma Selvarajah, Phil Mayhead and Rachel M Chalmers

O60 *Cryptosporidium* infection associated with maternal HIV among children under five years presenting with acute diarrhea in two Western Kenya Hospitals.

Patricia B Pavlinac, Grace John-Stewart, Jaqueline M Naulikha, Frankline O Onchiri, Elizabeth A Odundo, Benson O Singa, Barbra A Richardson, Donna M Denno and Judd L Walson

O61 Risk factors for giardiasis and parasite assemblages in North West England: a case-control study.

Corrado Minetti, Kenneth Lamden, John Cheesbrough, Caroline Durband, Nadine Randle, Katherine Platt, Steven Gee, Sarah J O'Brien and Jonathan M Wastling

O62 Six year follow-up of chronic fatigue and irritable bowel syndrome after giardiasis: a controlled prospective cohort study.

Kurt Hanevik, Knut-Arne Wensaas, Guri Rortveit, Geir Egil Eide, Kristine Mørch and Nina Langeland

O63 Prevalence, genetic diversity and risk factors of *Cryptosporidium* and *Giardia* infections among school children in Lebanon

Marwan Osman, Dima El Safadi, Sadia Benamrouz, Karine Guyot, Eduardo Dei-Cas, El Moukhtar Aliouat, Monzer Hamze, Fouad Dabboussi, Eric Viscogliosi, Gabriela Certad

# Scientific Programme

## FRIDAY 30<sup>th</sup> MAY

### CELL BIOLOGY

*Chair: Guy Robinson (UK)*

**8.30-9.10**

#### INVITED SPEAKER

O 64 *Giardia intestinalis*, a highly divergent eukaryote, has a noncanonical cell cycle.  
*Zacheus Cande (USA)*

**9.10-9.45**

#### ORAL PRESENTATIONS

O65 A new technique to study the *Giardia intestinalis* cell cycle.

Janet Yee, Chase Reaume, Kathleen Horlock-Roberts

O66 An unusual role for the giardial clathrin heavy chain?

Jon Paulin Zumthor and Adrian B Hehl

O67 Characterization of *Giardia lamblia* Cytochromes b5.

Steven P Rafferty, Robert Pazdzior, Alice Yang, Manon Couture and Janet Yee

**9.45-10.45**

#### POSTER SESSION AND FIKA (COFFEE)

**10.45-12.00**

#### ORAL PRESENTATIONS, CONT.

O68 Chromosomes and karyotype of *Giardia intestinalis*: individual cell analysis.

Pavla Tumová, Gerhard Wanner, Magdalena Uzlíková, Kristýna Marková and Eva Nohýnková

O69 Cytosolic iron/sulphur cluster assembly machinery in *Giardia intestinalis*.

Jan Pyrih, Martin Kolisko, Eva Martincová, Andrew Roger and Jan Tachezy

# Scientific Programme

O70 Molecular analysis of the mitosomal protein import in *Giardia intestinalis*.

Eva Martincová, Luboš Voleman, Vojtěch Žárský, Jan Pyrih, Jan Tachezy and Pavel Doležal

O71 Specific targeting of axonemal protein GAX to flagella of *Giardia intestinalis*.

Jan Tachezy and Jan Pyrih

O72 Unravelling the molecular basis for the elusive mitosomal protein import function in *Giardia lamblia*.

Samuel Rout, Jon Paulin Zumthor, Carmen Faso and Adrian B Hehl

**12.00-13.00**

LUNCH

## **GENOMICS, TRANSCRIPTOMICS & PROTEOMICS - I**

*Chair: Giovanni Widmer (USA)*

**13.00-13.40**

INVITED SPEAKER

O 73 *Cryptosporidium* AP2 proteins and evolution of gene networks in the Apicomplexa.

*Jessica Kissinger (USA)*

**13.40-15.15**

ORAL PRESENTATIONS

O74 Epigenetics and signal transduction in *Cryptosporidium parvum*.

Raymond Hui

O75 Label-free quantitative proteomics in *Giardia duodenalis*.

Samantha J Emery, Ernest Lacey and Paul A Haynes

# Scientific Programme

076 Oxygen metabolism and nitroheterocyclic drug resistance in *Giardia* – common themes and novel bioinformatics insights.

Brendan R E Ansell, Malcolm J McConville, Pasi K Korhonen, Michael J Dagley and Aaron R Jex

077 The Cre/loxP system in *Giardia lamblia*: Genetic manipulations in a tetraploid protozoan.

Carmen Faso, Petra B Wampfler and Adrian B Hehl

078 The genome and in vivo transcriptome of *Giardia muris*.

Jon Jerlström-Hultqvist, Feifei Xu, Elin Einarsson, Ásgeir Ástvaldsson, Lars Eckmann, Jan O Andersson, Staffan G Svärd

079 Investigation of waterborne outbreaks of giardiasis by whole genome sequencing.

Natalie Prystajecky, Clement Tsui, Will Hsiao, Anamaria Crisan, Jordan Ho, Patrick Tang, and Judy Isaac-Renton

080 The genome of *Spironucleus salmonicida* highlights a fish pathogen adapted to fluctuating environments

Jan O Andersson, Jon Jerlström-Hultqvist, Elin Einarsson, Ásgeir Ástvaldsson, Staffan G Svärd and Feifei Xu

**15.15-15.45**

**FIKA AND POSTERS**

**15.45-17.00**

**GENOMICS, TRANSCRIPTOMICS & PROTEOMICS - II**

*Chair: Omar Harb (USA)*

**ORAL PRESENTATIONS**

081 The genome is not enough: expanding our knowledge base through the genome sequencing of multiple clinical isolates of *Cryptosporidium* and *Giardia* from Scandinavia.

Cecilia Alsmark, Romanico Arrighi, Anders Allgardsson, Reza Advani, Erik Alm, Per Sikora, Jadwiga Winiecka-Krusnell, Steve Glavas, Charlotte Axén, Per Larsson, Karin Troell and Jessica Beser



# Scientific Programme

O82 The numbers game: development of a method to obtain high quality material from ALL *Cryptosporidium* and *Giardia* samples for genome sequencing.

Romanico B G Arrighi, Cecilia Alsmark, Jadwiga Winiecka-Krusnell, Reza Advani, Erik Alm, Steve Glavas, Jessica Beser and Karin Troell

O83 Transcriptional analysis of *Giardia intestinalis* encystation using RNA seq.

Elin Einarsson, Karin Troell, Malin Weiland and Staffan G Svärd

O84 Transcriptional profiling of *Giardia intestinalis* response to oxidative stress.

Showgy Y Ma'ayeh and Staffan Svärd

O85 Whole genome sequence analysis of a clinical isolate of zoonotic *C. parvum*: isolate UKPI

Rebecca A Guy, Kristin Elwin, Rachel A Chalmers and John E Nash

O86 Whole genome sequencing of *Cryptosporidium* spp. prepared directly from human clinical samples.

Stephen J Hadfield, Guy Robinson, Justin A Pachebat, Simon Cameron, Matt Hegarty, Kristin Elwin and Rachel M Chalmers

**17.00-17.40**

## **POPULATION GENETICS**

*Chair: Omar Harb (USA)*

O87 Cryptic genetic exchange in human giardiasis.

Juan David Ramírez, Theodore E Nash and Michael E Grigg

O88 Population structure of natural and propagated isolates of *Cryptosporidium parvum*, *Cryptosporidium hominis* and *Cryptosporidium meleagridis*.

Giovanni Widmer, Refaat Ras, Rachel M Chalmers, Kristin Elwin

**17.40-18.00**

## **CLOSING CEREMONY**

# Scientific Programme

**WEDNESDAY 28<sup>th</sup> MAY**

**17.30-19.30**

Workshop on:

Waterborne *Cryptosporidium* and *Giardia*: Regulatory and Legal Issues

*Organized by George D Di Giovanni, University of Texas-Houston School of Public Health*

*Whip-cracker: Lucy Robertson, Norwegian University of Life Sciences*

The purpose of this workshop is to provide a forum for sharing updates on regulatory and legal issues and associated research needs surrounding waterborne *Cryptosporidium* and *Giardia*. Waterborne outbreaks caused by *Cryptosporidium* and *Giardia* continue to occur worldwide despite continued advances in watershed management and water treatment. For the most part, national health and regulatory authorities and the global drinking water industry have not embraced current monitoring and characterization technologies to address the public health risks these pathogens pose. This workshop will include provocative presentations on different aspects of these issues and conclude with a candid and engaging roundtable discussion.

Water Monitoring...Achievable Improvements and Under Emphasized Needs

*Jerry Ongerth*

*Cryptosporidium* Slide Genotyping to Support Regulatory Monitoring

*George D. Di Giovanni*

Regulatory Response to Recent Drinking Waterborne *Cryptosporidium* Outbreaks in the UK

*Rachel Chalmers*

Waterborne *Cryptosporidium* Outbreak in Östersund, Sweden – Results, Actions and Response From Authorities

*Caroline Schönning and Micael Widerstrom*

Roundtable Discussion

*Workshop generously sponsored by Waterborne, Inc., New Orleans, USA and National Food Agency, Sweden*

## MINI-SYMPOSIUM: Childhood diarrhea-a global problem

- O1**      **The Global Enteric Multicenter Study (GEMS) of Diarrheal Disease in Infants and Young children in Developing Countries**

**Karen Kotloff<sup>1</sup>**

<sup>1</sup>University of Maryland, USA

# Oral Presentations

O2

**The interaction of enteric infections and malnutrition: consequences for child health and development**

**Bill Petri**<sup>1</sup>

<sup>1</sup>University of Virginia, USA

## O3 ***Cryptosporidium* work strategy development at the Bill and Melinda Gates Foundation**

**Ibrahim A Abdel-Messih Khalil<sup>1</sup>**

<sup>1</sup>Bill and Melinda Gates Foundation, Enteric and Diarrheal Diseases, Seattle, USA

There is increasingly growing evidence that demonstrates a high global burden of cryptosporidiosis among children in developing countries. At the same time, studies demonstrated a wide heterogeneity in prevalence. Burden estimates varied widely, even among studies from the same geographic region, possibly as a result of differences in study design, sample size, age range, HIV status, severity of disease, and sensitivity of diagnostic methods. Current diagnostic tests for cryptosporidiosis are suboptimal; requiring specialized tests that are insensitive at best. Interventions for treatment are also suboptimal with limited activity in normal hosts and no proven efficacy in controlled trials of AIDS patients. Further longitudinal studies that utilize advanced molecular methods are critical to characterize *Cryptosporidium* disease, pathogenesis, immune response and clinical outcomes are needed. Although there are promising therapeutic targets and drugs in development, methods for assessing their efficacy in vitro and in animals are not well standardized. Partial immunity following exposure suggests the potential of successful vaccination and several efforts for vaccine development are ongoing but till date, surrogates of protection are not well defined. These big knowledge gaps warrant strategic thinking towards better estimates for global burden, development of simple, inexpensive diagnostic tools and effective prevention and treatment interventions. The Bill and Melinda Gates Foundation is working to develop a strategy for cryptosporidiosis and discuss and share data on the global burden, diagnostics, therapeutics, and preventive strategies for cryptosporidiosis. This strategy will help to advance the understanding and address many areas of uncertainties in the field.

## FOOD- AND WATERBORNE TRANSMISSION

O4

Foodborne and waterborne transmission of *Cryptosporidium* and *Giardia*

Lucy J Robertson<sup>1</sup>

<sup>1</sup>Parasitology Laboratory, Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine and Biosciences, Norwegian University of Life Sciences, Norway

The importance of waterborne transmission of *Cryptosporidium* and *Giardia* has long been recognized, but foodborne transmission is slowly creeping up the agenda. An FAO/WHO risk ranking exercise suggested that *Cryptosporidium* could be considered the 5<sup>th</sup> most important of the various parasites that may be transmitted by food, but *Giardia* was ranked as considerably less important in position 11.

Although waterborne outbreaks of cryptosporidiosis occur more commonly than waterborne outbreaks of giardiasis (presumably largely due to the superior longevity of the oocysts, although other factors may play a role), with respect to foodborne outbreaks the predominance of *Cryptosporidium* over *Giardia* is even more marked.

With respect to this, several questions arise including: is food an important but under-estimated vehicle of infection for either or both these parasites? is it easier to detect foodborne outbreaks of cryptosporidiosis than of giardiasis? Regarding the potential for food contamination, what is the importance of these parasites as zoonoses? Is the survival of parasites in or on foods an important comparative factor?

In an attempt to answer some of these questions, this presentation considers the relative importance of food (and different food matrices) as vehicles of infection for both *Cryptosporidium* and *Giardia*, and also compares risk factors with those for waterborne transmission – about which we know considerably more.

# Oral Presentations

## O5 Essentials of Environmental Monitoring for *Cryptosporidium* and *Giardia*

Jerry E Ongerth<sup>1</sup>

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Waterborne outbreaks of giardiasis and cryptosporidiosis beginning from the 1960's-1980's, and the serious consequences of cryptosporidiosis in the early years of the AIDs epidemic led to global monitoring of these organisms in water. The means of monitoring is challenging, time-consuming, and expensive. And, resulting data are used to judge the health risk specific to the water source. A common method is USEPA 1622-1623. It has been applied widely and extensive data indicates that its performance is effective with recovery efficiency from fresh water over a range of quality typically for *Cryptosporidium* from 30-50% and for *Giardia* from 40-60%. Yet in widespread monitoring required by regulatory agencies and in large-scale surveys the vast majority analysis results are zeros. On the other hand the literature provides ample evidence that both organisms are virtually universally present in fresh surface waters worldwide and that levels should be readily detectable by effectively applied current methods. The predominance of negative results has masked the importance of measuring and reporting concentration rather than raw numbers/volume, without accounting for the recovery efficiency that is heavily dependent on water quality...the "matrix effect".

This presentation will include recent data demonstrating the controlling effect of the sample volume selected in monitoring on the ability to define the ambient concentrations of *Cryptosporidium* and *Giardia*. The quantitative effect on data interpretation of routinely measuring and applying recovery efficiency to express actual concentrations will be described using both previously published and recent data. Finally, two extensive data sets produced by USA water utilities under government regulations: 1) the Information Collection Rule (ICR) Supplemental Survey in 2000; and 2) the Long Term 2 Enhanced Surface Water Treatment Rule (LT2) in 2012, will be used to illustrate the virtually universal occurrence of these organisms in surface water of virtually any characteristics. Conclusions will be drawn from the data and analysis showing that the concentrations both organisms are readily definable using currently accepted methods. Essential requirements include collection and processing of sufficient sample volume, and measurement of recovery efficiency with every sample to calculate concentration. The effect on risk estimates of including recovery efficiency is typically a factor of from 2 to 10.

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Handling zoonotic diseases demands a good collaboration between involved authorities. *Cryptosporidium* as a cause of gastrointestinal disease was brought to the fore in Sweden by two large waterborne outbreaks 2010-2011, involving nearly 50,000 people. The combination of a low infectious dose, high oocyst excretion by infected individuals, oocysts already infective at excretion and hardy oocysts surviving long periods in the environment and withstanding treatment with disinfectants, make *Cryptosporidium* an important pathogen to acknowledge for public health. As part of a larger collaboration project; the Swedish Board of Agriculture, the National Food Agency, the Swedish Institute for Communicable Disease Control, the National Board of Health and Welfare and the National Veterinary Institute have worked out a strategy for handling of human *Cryptosporidium* infection. The strategy identifies different sources and paths of infection and especially important measures to decrease the risk of human *Cryptosporidium* infection. It was mainly developed for the involved authorities but is available for other authorities, the industry and the health care system. We will briefly present the importance of different infection sources and paths of human *Cryptosporidium* infection (e.g. drinking and recreational water) in Sweden, how monitoring of these sources is performed and the responsibilities of producers and authorities to ensure safe products and handling of outbreaks. The focus of the presentation will be on identified knowledge gaps and important measures that should be taken during the following five years.



## 07 $\mu$ AQUA – a universal microarray for the detection of waterborne pathogens

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The threat of waterborne diseases is predicted to increase in the future. To avoid outbreaks and ensure safety, water quality needs to be continuously monitored. A universal method for rapid and cost-efficient detection of waterborne pathogenic microbes has been sought after for many decades. Traditional methods usually are laborious, require high levels of expertise and typically focus on only one or a few organisms at once. As part of the interdisciplinary and international project  $\mu$ AQUA, we developed a universal microarray chip, equipped with species-specific oligonucleotide probes targeting protozoa, bacteria, viruses and cyanobacteria. The array is designed to include a hierarchical approach, with probes targeting higher clades to increase specific detection of pathogens.

Our contribution to the project is the designing and testing of probes that target pathogenic members of the protozoa *Cryptosporidium*, *Giardia*, *Naegleria* and *Entamoeba* in environmental water samples. Critical focus points include obtaining and processing samples for the extraction of high quality nucleic acids and the validation of the array signals with field samples using current bestavailable techniques. The project also involves monitoring of different types of waters in Europe, including three distinct Irish rivers which drain agricultural intense and urbanised catchment areas, providing water from different environmental conditions. The abundances of *Cryptosporidium* oocysts and *Giardia* cysts in the Irish rivers have been assessed monthly using the EPA method 1623. The greatest numbers of *Cryptosporidium* oocysts were found early in spring and autumn, mainly in the agriculturally intense catchment, but none of these were characterised as human pathogens.

08

## Selection of DNA aptamers for the detection of *Cryptosporidium parvum* in foods

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Aptamers are single-stranded oligonucleotides that fold into specific three-dimensional shapes and are capable of binding strongly and selectively to target cells or molecules. They offer advantages over antibodies, including reduced cost, ease of production and modification, and improved stability. This novel technology has not yet been exploited for the detection of foodborne protozoan parasites, for which existing detection methods are limited due to lack of enrichment steps, presence of inhibitors, and low parasite concentrations. The main objectives of the present study, therefore, were to generate and screen DNA aptamers for their ability to selectively bind to the oocyst wall of *Cryptosporidium parvum*, and to use the aptamers to detect the presence of *C. parvum* oocysts on foods. Random DNA sequences from large libraries were screened using an in vitro process known as systematic evolution of ligands by exponential enrichment (SELEX) for their ability to bind strongly and specifically with the oocyst wall of *C. parvum*. SELEX involves repetitive rounds of two processes: (i) partitioning of aptamers from non-aptamers using an affinity method, and (ii) amplification of aptamers by the polymerase chain reaction. In the present study, a total of twelve rounds of selection led to the recognition of a number of promising aptamers with very high affinity for *C. parvum* oocysts. Using flow cytometry, these aptamers were found to bind to *C. parvum* oocysts with an affinity in the low nanomolar range. As compared to a non-specific DNA control sequence, the *Cryptosporidium*-binding affinity of the aptamers was very much higher. A variety of biosensor platforms are currently being tested for their effectiveness in detecting parasite-bound aptamers. The sensitivity and specificity of labelled aptamers in detecting *C. parvum* oocysts on spiked foods will then be determined, and a comparison of the detection and recovery of *C. parvum* oocysts on foods, such as leafy greens, using DNA aptamers with that of conventional methods such as microscopy and PCR-based methods will also be performed. It is anticipated that DNA aptamers could provide a rapid and cost-effective alternative to antibodies in the detection of *Cryptosporidium* oocysts on foods.

09

## Wastewater treatment with *Moringa oleifera* seed extract: Impact on turbidity and sedimentation of *Cryptosporidium parvum* oocysts

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The use of low quality water for irrigation in agriculture is common practise in many countries due to limited freshwater resources. Pathogens may contaminate vegetables when faeces polluted water is used for irrigation. A laboratory study was carried out to investigate the effect of a coagulant produced from seeds of the *Moringa oleifera* tree (MO) in reducing *Cryptosporidium parvum* oocysts and turbidity in wastewater. To a total of 5 x 12 glass jars containing 500 ml wastewater samples from a Danish treatment plant,  $1.2 \times 10^6 \pm 1.2 \times 10^5$  oocysts L<sup>-1</sup> were added. To half of the wastewater samples 8 ml L<sup>-1</sup> of a 5% w/v MO seed extract was added, while the remaining wastewater samples were left untreated. The samples were stirred slowly for 20 min and subsequently left to settle for 15, 30, 45, 60 or 90 min, with six replicate glass jars representing each time point. In both treated and untreated wastewater, most oocysts settled within the first 15 min, while the sedimentation was negligible between 15 and 90 min. Nevertheless, MO seed extracts were effective in reducing the number of *C. parvum* oocysts significantly ( $p=0.026$ ) by 38% in the interval 15 to 90 min as compared to a 0.02% reduction in the untreated wastewater. Furthermore, the number of oocysts L<sup>-1</sup> was significantly ( $p<0.005$ ) lower in the treated wastewater at all 5 sampling times compared to untreated wastewater. The turbidity was reduced to  $9.8 \pm 1.8$  NTU (i.e. 95% reduction) in the treated water, which was significantly ( $p<0.0001$ ) lower than the lowest turbidity values of  $57.7 \pm 4.2$  (i.e. 72% reduction) seen in the untreated water. MO seeds are readily available in many tropical countries where the tree is common, and our results document that the seed extract may be used by farmers for treatment of different types of surface water. Yet, the study did not succeed in removing all oocysts from the wastewater after treatment, which was probably caused by an imbalance between the relative concentration of oocysts and MO seed extract with more oocysts present than the seed extract could adhere to. However, treatment of wastewater with MO seed extract significantly improved the water quality with regard to number of oocysts present and turbidity of the water. Further experiments with water samples containing fewer *C. parvum* oocysts are needed to establish whether MO seed extract can be used to effectively treat and obtain safe water free of *C. parvum* oocysts as well as other protozoan parasites.

## O10 Investigation into a *Cryptosporidium* Outbreak linked to a Swimming Pool

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In June 2013 a new swimming pool was opened in Rolleston, Canterbury, NZ. It serves a large rural population, with a significant amount of sheep and dairying occurring in the district served by the swimming pool. On Aug 30th an alert was raised on the NZ epidemiological surveillance network, as 4 cases of cryptosporidiosis were notified for the area within two days. A watching brief was kept, and by Monday 9th Sept there were 7 confirmed cases, six of whom had swum at the Aquatic Centre. Following discussions with the pool, it voluntarily closed and an extensive disinfection of the pool area was undertaken and all water in the pool was disposed off.

The number of final number of cases of cryptosporidiosis was estimated at 22, but several of these had family members ill that were not tested. Phone interviews were conducted on 18 cases and from the information gathered 15 had contact with the pool or had family members that did. Faecal samples were sent to Massey University for PCR and sequencing. While analysis is still on-going, to date the outbreak cases were determined to be *C. hominis* subtype 1bA10G2. *C. parvum* was the genotype isolated from the non-outbreak cases during the same time frame.

Investigations of the pool and its operations found a number of operational practices that were not sufficient. A number of new practices were put in place including new signage asking people not to swim if they had diarrhoea in the previous 10 days. A community information campaign was undertaken by the council in conjunction with the local public health unit. Following cleaning of the aquatic centre and refilling the pool water, testing was undertaken of each of the pools in the centre. All water samples were negative for cryptosporidium. Following closure of the centre for just under three weeks it pool reopened to the public. This outbreak raised several questions in relation to the design of swimming pools and the increased awareness needed regarding diarrheal infections in the community. Investigations required the collaboration and co-ordination of several agencies and strengthened their linkages and understanding of each of their roles.

## O11 Swimming pool outbreaks outbreak of *Cryptosporidium* across England 1988 to 2012

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There have been 98 swimming pool or water feature related outbreaks of gastroenteritis/ infectious intestinal disease reported in England and Wales over the period 1988 to 2012. The majority of outbreaks (90/98; 92%) involved *Cryptosporidium*, usually alone but occasionally with another pathogen, with other outbreaks caused by VTEC O157 (3), *Giardia* (3), *Campylobacter* (1) and a suspected viral infection (1). An increase in the number of *Cryptosporidium* outbreaks over time is related to improved surveillance within regions, and active investigation including detection of oocysts in water and typing through the Cryptosporidium Reference Unit. Pool outbreaks caused by *Cryptosporidium* are strongly seasonal, mainly occurring in the summer and autumn, and are predominantly caused by *C. hominis*. A majority of outbreaks involve less than 20 reported cases (median 10; mean 17.5; range 2 to 122). Cases are mainly in children. The common occurrence of small outbreaks linked to swimming pools suggests that cryptosporidiosis is an inherent problem in such settings. The construction, design and management of conventional treatment (flocculation, filtration, chlorination) is inadequate to prevent outbreaks and there is a need for secondary treatment (UV) to reduce exposure of swimmers to viable oocysts as well as industry engagement, swimmer and operator education.

## **O12      An outbreak of *Cryptosporidium parvum* across England & Scotland associated with consumption of fresh pre-cut salad leaves**

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An outbreak of over 300 cryptosporidiosis cases caused by *Cryptosporidium parvum* gp60 subtype IIaA15G2R1 occurred in May 2012 and was associated with consumption of bagged salad from a single national retailer. The outbreak occurred over a two week period, and in many regions of England and Scotland. Analyses included rapid case-case analysis in one region comparing age distribution and exposure between different years, national case-case descriptive epidemiology comparing cases with previous years, hypothesis generation using an extended questionnaire for four cases in each of four regions, a case-control study with 74 cases and 74 controls across four English regions and Scotland and subtyping data. Results indicated that the outbreak was caused by transient contamination of the salad supply chain. Reference laboratory typing was crucial to the investigation, but there is still a need to be able to better separate the outbreak strain from background isolates. This is the largest reported outbreak of cryptosporidiosis associated with food, and identifies a number of problems with the detection, investigation, control and prevention of cryptosporidiosis linked to fresh produce. This includes limitations in traceback, possible issues with irrigation and washing, fear of litigation and publication and on-going problems with industry denial. Within Europe there is also a need to improve diagnostic, reference and typing services.

## INFECTION IN ANIMALS AND ZOO NOTIC ASPECTS

### O13 *Cryptosporidium* infection and associated calf-level risk factors in French dairy calves Carine

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**Background:** This study was conducted to determine the prevalence and the risk factors for *Cryptosporidium* infection in calf neonates on farms in an area of intensive dairy farming (Normandy, western France).

**Material and methods:** Fecal samples were randomly collected from 968 calves, ranging from 7 to 21 days old, on 97 farms during the calving season between July 2010 and September 2011. Up to 10 calves were sampled per farm. Samples were examined using the Heine technique. *Cryptosporidium* oocyst shedding was scored semi-quantitatively (0 to 5). A questionnaire designed to gather information on calf-level care and management was conducted. Mortality rates were obtained from the French national registration database. Multivariate analysis of the potential risk factors for oocyst shedding was first conducted using a logistic regression and fixed effects were then included in a generalized estimating equation.

**Results:** 402 of 968 calves (41.5%) were positive and 25.1% had a shedding score of >2. Seven of the 97 farms (7%) were negative for all calf fecal samples. At the time of collection, 375 calves (39%) had diarrhea. The prevalence of diarrhea was strongly correlated with the score of oocyst shedding ( $p < 0.0001$ ). The mortality rate at 90 days was significantly higher in calves with high combined scores of diarrhea and shedding. Factors associated with moderate oocyst shedding (Heine score more than 1) included the distribution of colostrum using a bucket (OR=1.4; 95% CI 1.0-1.9). The use of fermented milk following colostrum was protective (OR=0.3; 95% CI 0.2 - 0.6). Factors associated with high shedding (Heine score more than 3) were the Normande breed (OR =2.0; 95% CI 1.0, 4.0) and the distribution of colostrum using a bucket (OR=1.9; 95% CI 1.3-2.7). Treatment with halofuginone lactate and feeding fermented milk were protective (OR=0.3; 95% CI 0.1-0.7; OR=0.1; 95% CI 0.0- 1.1, respectively).

**Conclusions:** The results show that *Cryptosporidium* infection is widespread among calves less than 21 days of age in western France dairy herds. In addition, *Cryptosporidium* appears to be one of the primary causes of diarrhea and increases the risk of mortality. This study identified some causal and protective calf-level factors, however further investigations are necessary to determine the appropriate measures that farmers and veterinary practitioners should take to control *Cryptosporidium* infection in dairy calves.

## O14 *Cryptosporidium* infection in beef calves in Sweden

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*Cryptosporidium* spp. are clinically important pathogens causing gastrointestinal disease and diarrhea in a variety of species including humans and cattle. One of the species infecting cattle, *C. parvum*, is zoonotic and can be transmitted between cattle and man. This species is primarily found in preweaned calves. Because of questions surrounding the extent of *C. parvum* shedding into the environment from cow-calf herds in Sweden and the potential zoonotic implications this study was implemented. The aims were to investigate how common *Cryptosporidium* infection is in calves in Swedish cow-calf herds and to explore which species and subtypes that occur. Thirty herds were enrolled in the study. Each herd was visited once either during April to June 2012 or April to May 2013. Faecal samples were collected from all calves younger than 3 months, and information about the herd and management routines were registered. The samples were cleaned and concentrated by saline-glucose flotation, stained with monoclonal anti-*Cryptosporidium* antibodies and analysed for presence of oocysts by epifluorescence microscopy. The lower detection limit of this method is 400 oocysts per gram faeces. Molecular analysis of the *ssrRNA* and *GP60* loci was performed.

A total of 332 calves from 1 to 90 days of age were sampled and *Cryptosporidium* oocysts were detected in 122 (37%) of them. Positive calves were identified in 29 (97%) herds and the withinherd prevalence varied between 6% and 75% (median 42.3). Statistical analysis identified two factors, routines for cleaning calf pens and number of cows in calving pens, as associated with *Cryptosporidium* infection. However, the sensitivity and specificity and prediction probability of the model was low, indicating that other important factors were missed.

*C. bovis* was the species most commonly found. *C. parvum* was detected in 28 calves (8%) in 10 herds. Nine calves had a mono infection with *C. parvum* whereas mixed infections with *C. bovis/C. parvum* or *C. ryanae/C. parvum* were found in the other 19 calves.



**O15**      **Detection and molecular epidemiology of *Giardia duodenalis* and *Cryptosporidium* spp. in asymptomatic adult cattle in Northern Spain**

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Infections by the protozoan parasites *Giardia duodenalis* and *Cryptosporidium* spp. are a common cause of intestinal disease in cattle. Giardiasis and cryptosporidiosis affect primarily calves, where severe diarrhoeal episodes have been linked to weight loss and delayed growth, which in turn leads to substantial economic losses. Although adult individuals usually present lower prevalence and disease burden rates, they may serve as natural reservoir of infection to younger animals and source of environmental contamination with (oo)cysts of these pathogens. Here we present epidemiological and molecular data on the presence of *G. duodenalis* and *Cryptosporidium* spp. infections in asymptomatic adult (>2-year-old) cattle from the province of Álava, North of Spain. A total of 363 faecal samples were collected from four farms from November 2011 to December 2012. Detection of *Giardia* was achieved using an in-house real-time PCR targeting the small-subunit (SSU) ribosomal DNA.

*Giardia*-positive samples were analysed using a semi-nested *gdh* PCR and a nested  $\beta$ -giardin PCR (expected amplicons of 432 and 511 bp, respectively). A nested SSU PCR (expected amplicon of 516 bp) was used to detect the presence of *Cryptosporidium* spp. Restriction fragment length polymorphism (RFLP) analyses with appropriate restriction enzymes were performed on aliquots of the products from all PCR amplifications. *Giardia* assemblages and *Cryptosporidium* species were confirmed by sequencing both strands of the DNA. A phylogenetic analysis including reference sequences was conducted to investigate strain similarities among the obtained *Giardia* and *Cryptosporidium* isolates. Overall, the prevalences of *Giardia* and *Cryptosporidium* were estimated at 18.7% and 16.3%, respectively. Both parasites were detected in all four farms studied and were found infecting cattle of all the age groups considered with no statistically significant differences.

Fourteen *Giardia* isolates were characterized as Assemblage E (*G. bovis*), and an additional three as Assemblage F (*G. cati*). No dual infections were identified. Genotyping analyses of *Cryptosporidium* isolates are currently in progress. Our results clearly show that *G. duodenalis* and *Cryptosporidium* spp. infections are frequent in cattle from the province of Álava. Regarding *Giardia*, the facts that only assemblages E and F have been found circulating in bovines and both pose little significant threat to human health seem to indicate that cattle play a marginal role as reservoir of zoonotic disease. The extent of this hypothesis should be confirmed in future studies with larger animal (including calves) populations.

## O16 The role of adult cattle in the transmission and persistence of *Cryptosporidium*

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It has been suggested that adult cattle may be a source of infection with *Cryptosporidium* for neonatal calves. A few studies have been carried out to test this theory but many of them assumed that the presence of *Cryptosporidium* in the adult faecal samples (most usually by microscopy or sometimes ELISA – to detect *Cryptosporidium* antigens in faecal supernatant using an anti-*Cryptosporidium* antibody) meant that they were the source from which calves became infected.

However, the methods used were unable to distinguish between different *Cryptosporidium* species present and only confirm the presence of oocysts. Now that it is known that there are many different *Cryptosporidium* species and that cattle may be a host for at least four, the question of the source of *C. parvum* infection for neonatal animals needs to be revisited. A longitudinal study was conducted to assess the shedding of *Cryptosporidium* oocysts in adult cattle, to identify the species shed and to determine if these adult cattle were the source of infection for their calves. Faecal samples were collected from 30 in-calf adult dairy cattle (heifers and cows) three times per week for up to ten weeks pre-calving. After calving faecal samples were then collected from the calves for at least six weeks three times per week and again at 3 and 6 months. Samples were tested for *Cryptosporidium* species and genotypes by nested species specific multiplex PCR (nssm-PCR) amplification and sequencing.

Two hundred and three samples were collected from the dams and 456 from calves. DNA has been extracted from all samples (using a new technique developed for oocyst concentration and DNA extraction from adult cattle faecal samples). Preliminary results from nssm-PCR of adult samples has shown some positives for *C. parvum*, *C. ryanae* and *C. bovis*, so far only *C. parvum* has been detected in the calf samples. Completed results for the speciation and genotyping of all adult and calf samples will be presented and the role of adult cattle as a source of *Cryptosporidium* infection for their calves will be discussed.

## O17 Transmission dynamics of *Cryptosporidium parvum* between beef farms in two regions of Scotland

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Transmission of *Cryptosporidium parvum* between farms can potentially occur via two routes: 1. over long distances, spread by movements of infected livestock, or 2. locally, spread by fomites, wildlife reservoirs or shared watercourses (indirect transmission) or by contact between animals at contiguous boundaries (direct transmission). In order to investigate the transmission dynamics of *C. parvum* at local and regional levels, in response to anecdotal reports of increased virulence of cryptosporidiosis, we conducted a crosssectional survey of beef farms in two regions of Northeast Scotland. Twenty seven farms in Aberdeenshire and Caithness, associated with 5 veterinary practices, were sampled in spring 2011. Results showed that 92/190 calves and 21/27 farms sampled were positive for *C. parvum*, as identified by sequence analysis of the 18S rRNA gene. These positive isolates were then genotyped by multilocus fragment typing (MLFT).

Markers selected were MM5, MM18, MM19, TP14, MS1, MS9 and alleles were assigned based on size of fragments amplified. In addition, a region of the GP60 gene was sequenced and here alleles were assigned based on GP60 sequence subtype. In total, 78 samples from 19 farms were successfully typed at all 7 markers. 14 multi locus genotypes (MLGs) were identified. Most calves (74/78) and farms (14/19) had a single MLG identified. Most MLGs (8/14) were only detected on one farm but some “common” MLGs were found on multiple farms. Preliminary analysis of molecular data has identified evidence for local transmission of oocysts: the presence of the same or similar genotypes is significantly associated with Euclidean distance between holding addresses. However, this is a simplification of the system as animals may be located far from the holding address and farms are often fragmented into parcels of grazing land. In addition there may be natural barriers to local transmission including waterways and road systems. A small subset of the farms was revisited in the two following calving seasons (Spring 2012 and 2013). The MLGs remained stable in the majority of cases although on some farms the molecular profile was more dynamic, possibly reflecting biosecurity practices such as livestock movements onto farms.

## O18 Age related susceptibility of pigs to *Cryptosporidium scrofarum* infection

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Piglets from 4 to 8 weeks of age originated from a *Cryptosporidium*-free research breed were orally inoculated at 4, 5, 6, 7, or 8 weeks of age with  $1 \times 10^6$  oocysts of *Cryptosporidium scrofarum*. The number of shed oocysts per gram of faeces served to describe the infection intensity and prepatent period. In addition, daily faecal samples, and tissue samples of various parts of small and large intestine obtained at 30 days post inoculation were examined for *C. scrofarum* small subunit ribosomal RNA gene sequences by PCR and sequence analysis. The piglets inoculated at 4-weeks of age remained uninfected, whereas 5-week-old and older animals were fully susceptible with a prepatent period ranging from 4–8 days. Susceptible pigs shed oocysts intermittently, and shedding intensity, which reached a mean maximum of 6,000 oocysts per gram, did not differ significantly among infected animals. This study demonstrates that pigs become susceptible to *C. scrofarum* infection as late as 5-weeks of age. The mechanisms of age related susceptibility remain unknown. This study was funded by projects of the Ministry of Education, Youth and Sports of the Czech Republic (LH11061) and the Grant Agency of University of South Bohemia (022/2010/Z and 011/2013/Z)

## O19 *Giardia* and *Cryptosporidium* in coyotes and wolves in northwestern Canada

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Wolves (*Canis lupus*) and coyotes (*Canis latrans*) were harvested by trappers, hunters, and wildlife personnel from British Columbia (BC), Saskatchewan (SK), and Manitoba (MB), all south of 60°N, and the Northwest Territories (NT) (north of 60°N) in northwestern Canada between 2009 and 2011. One gram of rectal feces or distal colonic contents was recovered from each individual. *Giardia* cysts and *Cryptosporidium* oocysts were concentrated by centrifugation in a sucrose gradient and detected and quantified using a commercial immunofluorescent assay. DNA was extracted from the sucrose-purified cyst suspension and PCR assay and sequence of a 292 bp region of the 16S-rRNA ribosomal unit of *Giardia* was used to identify the assemblage(s) present in each sample. Prevalence of *Giardia* was highest in wolves from the NT (32% of 73) and lower in SK/MB wolves (15% of 19) and coyotes in BC (8% of 26). Conversely, prevalence of *Cryptosporidium* was higher in BC coyotes (31% of 26) and SK/MB wolves (30% of 19) than in NT wolves (19% of 73). For all wild canid populations, fecal shedding (cysts/oocysts per gram of feces) was 3-6 times higher for *Giardia* than *Cryptosporidium*, suggesting more potential for environmental contamination with *Giardia*. These findings suggest that *Giardia* is particularly well adapted to northern climates. Finally, zoonotic Assemblage B *Giardia* dominated in all populations, with only two mixed infections with B and C (dog-specific genotype) observed. Based on this and concomitant investigations of domestic dogs (*Canis familiaris*) in northern communities, zoonotic genotypes of *Giardia* dominate in wild and domestic canid populations of the Canadian North. Therefore, people may be key in the dissemination and transmission of this parasite and knowledge about anthropogenic cycles is important for assessing and managing risks to animal and public health.

O20

## Occurrence of *Giardia* and *Cryptosporidium* in captive chimpanzees (*Pan troglodytes*), mandrills (*Mandrillus sphinx*) and wild Zanzibar red colobus monkeys (*Procolobus kirkii*)

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The transmission of pathogens between human and non-human primates is facilitated by their close taxonomic relationship, posing a threat to both public health and wildlife conservation. The aim of this study was to identify the occurrence of *Giardia duodenalis* and *Cryptosporidium* spp. in nonhuman primates and use molecular characterisation to determine whether they had zoonotic or anthroozoonotic potential.

Using direct immunofluorescence, *Giardia* cysts were identified in faecal samples from 5.5% (5/90) of wild-born captive chimpanzees (*Pan troglodytes*) and 0% (0/11) of wild-born captive mandrills (*Mandrillus sphinx*) at Tchimpounga Chimpanzee Rehabilitation Centre, Congo; 0% (0/10) of captive chimpanzees at Kristiansand Zoo, Norway; and 0% of faecal samples (n=49) from wild Zanzibar red colobus monkeys (*Procolobus kirkii*).

*Cryptosporidium* oocysts were not detected in any of the samples. Two samples from chimpanzees at Tchimpounga contained objects with a morphology similar to that of *Cryptosporidium* oocysts, however due to the limited number of these objects, negative DAPI staining, and negative PCR, identification of *Cryptosporidium* could not be confirmed. PCR was performed on the *Giardia*-positive samples, using standard primer sets directed at 3 genes (glutamate dehydrogenase, triose phosphate isomerase and  $\beta$ -giardin), with 4/5 (80%) of these samples testing positive at one or more gene. Sequencing demonstrated that these chimpanzees were infected with Assemblage B. These results indicate that in these primate groups, in which interactions with humans and human environments are quite substantial, *Giardia* and *Cryptosporidium* are rare pathogens, and that in chimpanzees, *Giardia* has a zoonotic potential.

**O21**      **Molecular characterization and genotyping of *Cryptosporidium* spp. in chicken and turkeys in Germany**

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*Cryptosporidium* is one of the most prevalent enteric protozoan parasites infecting a wide range of host species, including mammals, birds, reptiles and fish. The disease is relatively neglected in poultry, although it can cause respiratory and/or intestinal disorders. In addition, infestation can compromise the efficiency of vaccination against viral diseases. Furthermore, *Cryptosporidium* can be transmitted to humans. The aim of this study is the molecular characterization and genotyping of *Cryptosporidium* spp. detected in faecal samples from broilers and fattening turkey flocks in Germany. Extraction of the DNA was done using the NucleoSpin® Soil kit (Macherey-Nagel, Germany). A 830 bp 18S rDNA fragment was amplified by nested PCR. Positive samples were subjected to direct automated DNA sequencing. A total of 19 of the 256 samples (7.4%) were positive for *Cryptosporidium* spp., 6.3% (10/158) in broilers, 9.2% (9/98) in turkeys. *C. parvum* was detected in 3.8% (6/158) of broilers and 7.2% (7/98) of turkeys, while *C. baileyi* was detected in 1.9% (3/158) of the broilers and in 2% (2/98) of turkeys.

*C. meleagridis* was detected in one of the 98 turkey samples. These results indicate that *Cryptosporidium* is common among broilers and turkeys in Germany, despite high hygiene standards, and should not be overlooked as a pathogen in poultry. Further studies to determine transmission pathways and the effect of the disease on production of poultry are required.

## O22 Characterisation of *Cryptosporidium* in Fish

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Current knowledge of the epidemiology, taxonomy, pathology and host specificity of *Cryptosporidium* species infecting piscine hosts is limited. In addition to *Cryptosporidium molnari*, a total of 13 additional species/genotypes have been identified in fish using molecular tools; piscine genotypes 1 to 8, *C. parvum*, *C. hominis*, *C. xiaoi*, *C. scrofarum* and rat genotype III have been identified. Understanding the potential impact and pathology of these parasites on fish and the potential zoonotic risks they pose is important. The identification of novel and zoonotic species of *Cryptosporidium* in piscine hosts and their phylogenetic relationships will be discussed.



## O23 Identification of *Cryptosporidium* species in fish from the Lemman Lake in France

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Only few data are currently available regarding the characterization of *Cryptosporidium* species and genotypes in natural aquatic environments and more particularly in edible fish. The aim of our study was to evaluate the prevalence of *Cryptosporidium* spp. in fish from the Lemman lake (France), the largest freshwater reservoir in western Europe. In total, 45 fish were collected from fisheries suppliers at Thonon-les-Bains, at the border of the lake, in November 2011 (fall) and April 2013 (spring). Stomach and intestinal epithelial cells were scrapped off and placed into tubes containing the fixative reagent, RCL2. After DNA extraction, detection and identification of *Cryptosporidium* was performed by PCR-sequencing using degenerated primers targeting the 18S rRNA gene. Subtype identification of *C. parvum* was subsequently done by amplification and sequencing of the GP60 gene. Histological examination of tissues was also conducted. The presence of *Cryptosporidium* was detected in 9 fish collected in fall, leading to a global prevalence of 21.95% distributed as follows: eight *C. parvum* specimen and one *C. molnari*. Among the intestinal samples, five of them were positive for *C. parvum*. Among the stomach samples, one was positive for *C. molnari* and three for *C. parvum*. All fish collected in spring were negative for *Cryptosporidium*. Moreover, four new fish species were identified as hosts of *Cryptosporidium* spp.: Arctic char (*Salvelinus alpinus*), Northern pike (*Esox lucius*), European whitefish (*Coregonus lavaretus*) and European perch (*Perca fluviatilis*). A moderate inflammation reaction was present in some of the fish as shown by histological analysis. The detection of zoonotic species of *Cryptosporidium* in fish is of special interest and should be explored further. In addition, the presence of *Cryptosporidium* should be considered as a sentinel for environmental contamination by human or animal faeces. This work was supported by the French National Research Agency (grant No. ANR-10-ALIA-004) and the regional pole of competitiveness AQUIMER.

## DIAGNOSTIC AND TREATMENT

### O24 Implications of molecular detection on surveillance of intestinal parasites

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In recent years there is an increasing trend toward the routine use of rapid immunoassays and PCR based methods to detect protozoan intestinal parasites in stool samples, particularly in larger laboratories processing larger numbers of samples. One of the reasons to use these techniques instead of conventional, microscopy based, techniques is the possibility to use automatic DNA extraction and multiplex real time PCR, which reduces the hands-on time and the costs. By doing so the laboratories not only changed the sensitivity and specificity of the detection but also changed the algorithm for a diagnostic request. E.g. *Cryptosporidium* was usually only tested on special request in young children or immunocompromised patients – due to the fact that a specific staining was necessary. In a multiplex PCR it is part of the package and results are sent for all patients. In The Netherlands the number of laboratories that implemented multiplex PCR testing for intestinal parasites in routine clinical practice increased to 30 labs in the last 10 years. In most of these laboratories the testpanel includes a multiplex PCR to detect specific DNA of *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Entamoeba dispar* and *Dientamoeba fragilis* simultaneously. There is, however, a difference between the labs regarding the interpretation of positive PCR results; whilst in a number of laboratories a positive confirmatory test (using conventional microscopy either or not combined with an immunoassay to detect copro-antigens) is performed prior to actually reporting the test results; other laboratories do not. The clinical implication of a positive PCR result (including high CT values indicating low DNA load) as such is not always clear and there is no consensus how to report and interpret the results to clinicians and public health officers. This can cause problems for public health: how to act upon an increase of incidence / prevalence due to PCR positivity. Is there a real outbreak or is it due to a change of methods, due to a change in algorithm, or all three? Several “outbreaks” of *Dientamoeba* eg have resulted in worried parents and personnel in primary school and time consuming efforts to find a source by PH officers. In order to be able to interpret the increase in numbers of positive *Cryptosporidium* cases in the Netherlands in 2012 and 2013 it appeared to be important to have access to detailed information about changes of the laboratory management as compared to previous years.

**Conclusion:** It is of great importance to consider the implications of changes in diagnostic protocols or algorithms for patient management prior to implementing a new detection system for intestinal parasites.

## O25 Introduction of a *Giardia* typing service in Wales (UK)

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There are approximately 150 to 200 reported cases of giardiasis in Wales per year (4.9 to 6.5 cases per 100,000 population); however giardiasis receives relatively little public health attention, largely because the illness is considered to be travel-acquired and has a low socioeconomic burden. In June 2012 a *Giardia* typing service was introduced in Wales using a newly developed real-time Assemblage specific probe-based PCR method. Typing results were reported to health protection colleagues in real-time in order to inform infection control measures. In addition, to investigate further the epidemiology of *Giardia* in Wales, cases were interviewed by a local environmental health officer and an enhanced surveillance questionnaire was completed, providing information on possible risk factors for infection.

Between June 2012 and December 2013, 192 cases of giardiasis were typed. Enhanced surveillance questionnaires were available for 178 cases. Cases were equally distributed in males and females, with a median age of 36 years (range: 1 – 81). The majority of cases (91%) were from a white ethnic group, and were UK born (91%). Cases reported being ill for a median of 16 days (range 0-98 days), and 8% reported being admitted to hospital. Half of cases reported contact with companion animals and 15% had recently attended a zoo or farm. Thirty-nine percent reported using a swimming or paddling pool in the two weeks before onset. Twenty three percent reported a history of overseas travel in the previous two weeks. Of the 192 cases typed, 41 were Assemblage A and 144 were Assemblage B, 5 were mixed Assemblage A and B. Two cases were not typable. A comparison of the characteristics of cases with Assemblage A and B is currently being carried out.

026

## A puzzling relationship between albendazole metabolism and albendazole resistance in *Giardia duodenalis*

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Mebendazole and albendazole (ABZ) are used for the treatment of giardiasis and in massive campaigns of deworming children most commonly at suboptimal doses. ABZ resistance in *Giardia* has been increasingly reported and this may be related to the inhibition of oxidative stress, i.e. reactive-oxygen species (ROS) accumulation. In other models, it has been reported that ABZ is converted into oxidized sulfoxide (ABZ-SO) and sulfone (ABZ-SOO) metabolites through activity of monooxygenases and/or cytochrome P450-type (cyp) enzymes. In this work we determined by quantitative HPLC if *G. duodenalis* trophozoite clones susceptible (DMF) and resistant (RA) to distinct concentrations of ABZ (RA1.35, RA8 and RA250  $\mu$ M) were able to metabolize this drug into sulfoxide and sulfone. Both ABZ-SO and ABZ-SOO were detected in all drug-exposed clones but were detected at higher concentrations in the DMF-treated clone. However in subculture assays the viability of this clone was totally altered by higher concentrations ( $>3\mu$ M) of both ABZ-SO and ABZ-SOO than ABZ ( $\sim 0.3\mu$ M), suggesting that ABZ metabolism is a detoxifying mechanism in ABZ-susceptible parasites. Further assays by flow cytometry determined ROS accumulation with distinctive patterns in drug-exposed clones, by all three ABZ species. Upon ABZ exposure, the DMF-treated clone showed significantly higher ROS levels than RA clones and conversely the exposure to ABZ-SO and ABZ-SOO induced much higher ROS accumulation in RA clones as compared to the DMF treated clone. A mining in the GiardiaDB revealed the absence of a cyp homolog and the presence of two potential cyp-reductases (cypor) and at least one of these (GL50803\_91252) showed higher mRNA expression levels in RA clones suggesting a more active processing of ABZ. These data showed that the rate of ABZ metabolism is different between susceptible and resistant *G. duodenalis* trophozoites. ABZ is more active against drug-susceptible parasites while ABZ-SO and ABZ-SOO are cytotoxic for ABZ-resistant trophozoites. Yet paradoxically, ABZ-resistant clones can evade ABZ activity likely through cypor-related metabolism but the accumulation of ABZ-SO/ABZSOO needs to be blocked thus trophozoites could survive upon cytotoxic ABZ concentrations.

**O27**      **GiTox: a simple colormetric method for screening drug susceptibility in *Giardia duodenalis***

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*Giardia duodenalis* is a parasitic protozoan of vertebrates responsible for gastrointestinal illness worldwide. The anti-parasitic repertoire for giardiasis treatment is limited and, factoring in emerging resistance to the primary drug metronidazole, methods capable of both screening for novel compounds and monitoring resistance to known anti-giardials are essential. We have achieved a simple and reproducible colormetric method for screening *Giardia* growth in anaerobic culture conditions. *G. duodenalis* is grown in 96 well plates in TYI-S33 media in the presence of the chromogen, where confluent growth is accompanied by a change in colour from blue to yellow in 72 hours. This colour change can be measured spectrophotometrically to determine the anti-giardial activity of a compound at either a fixed concentration or across serial dilution to determine 50% inhibitory concentration (IC<sub>50</sub>). The reliability of this method was determined through comparison with IC<sub>50</sub> obtained using a haemocytometer to identify total number of viable cells across a range of strains of varying host and geographical origins as well as resistance status. We believe this method is easily applied as high-throughput assay for screening chemical libraries for novel anti-giardials, as well as a simple and robust method to measure metronidazole resistance in laboratory and field isolates.

O28

## **Elaboration of a vaccine to immunize pregnant cows to protect newborn calves from *Cryptosporidium parvum* infection**

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*Cryptosporidium parvum* causes moderate to severe neonatal diarrhea in dairy calves. Infection-induced dehydration and electrolyte imbalance can be life-threatening, and mortality, as well as economic losses, is a major concern in the bovine industry worldwide as in the province of Quebec in Canada. Our objective is to elaborate a multivalent subunit vaccine to immunize pregnant cows to induce production of hyperimmune colostrum which will protect, by passive immunization, newborn calves from frank cryptosporidiosis. To do so, we selected immunogenic parts of four *C. parvum* sporozoite/merozoite surface proteins, namely CP2, p23, gp45 and gp900. We cloned DNA sequences and expressed recombinant proteins using an *E. coli* expression system. Each purified recombinant protein was used to raise hyperimmune antiserum in rabbits. These antisera are highly sensitive and specific for the recombinant protein against which they were raised. We are now testing the ability of these four antisera (anti-CP2, anti-p23, anti-gp45 and anti-gp900) to inhibit *C. parvum* infection of Madin-Darby bovine kidney cells. Our hypothesis is that specific antibodies in these antisera will block sporozoite/merozoite attachment to and invasion of mammalian cells. Effect of antisera on cell growth has already been determined and a colorimetric assay is being optimized to allow dose response assays as well as comparison of the efficiency of single antiserum with combinations of antisera. In parallel, we are testing the ability of these antisera, given orally, to prevent oocyst shedding and severe clinical signs of cryptosporidiosis in interferon gamma receptor knockout mice. Pilot studies are ongoing and will be followed by double blinded controlled studies. The infection model has been optimized and preliminary results are very promising. We are expecting antisera-treated mice to have mild, if any, cryptosporidiosis clinical signs as well as a significant reduction in oocyst shedding. Currently, neither drug treatments nor vaccines are available to control bovine cryptosporidiosis. Therefore, there is an urgent need to find new preventive approaches to improve calves wellbeing and decrease calf mortality related to *C. parvum* infection and this project constitutes the first step in the elaboration of an efficient combination vaccine against bovine cryptosporidiosis.

**029 Efficacy of chitosan, a natural polysaccharide, against *Cryptosporidium parvum* development in infected hct-8 and caco-2 enterocytic cells**

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Cryptosporidiosis is a zoonotic disease caused by a protozoan parasite, *Cryptosporidium parvum*. In animals, it is considered as an economically important disease with clinical signs and death in young ruminants. The usual clinical course is acute diarrhea affecting animals from 1 to 3 weeks old. Today, no drugs are fully effective in the treatment of cryptosporidiosis in man and animals. Therefore the research for new therapeutic agents is crucial. We report here details of the adaptation of in vitro culture systems (HCT-8 and Caco-2 cell lines) for *C. parvum* to investigate the "anticryptosporidial" activity of drugs and the results obtained with two new molecules (Chitosan NAG and Chitosan Mix). Chitosan is a sugar that is obtained from the hard outer skeleton of shellfish, including crab and shrimp. It is used for medicine. Chitosan, a natural polysaccharide compound, has been found to be active against a variety of diseases including antimicrobial and antitumoral effects. We investigated the effects of Chitosan in our two in vitro models we established in the laboratory. Paromomycin, a classical drug used in veterinary medicine, was used as a positive control. Immunofluorescence technique was used for the identification and enumeration of the parasites. Our results showed a very significant reduction of viability of *Cryptosporidium* oocysts (>95%) after pre-incubation of 24h at 37°C with Paromomycin (P<0.001), Chitosan Mix and Chitosan NAG (P<0.001). On the other hand, Paromomycin, Chitosan Mix and Chitosan NAG inhibited significantly the development of *C. parvum* in HCT-8 and Caco-2 cell lines (P<0.005). These effects are dosedependent. Synergic effects were obtained when Chitosan Nag treatment was associated with Paromomycin. In conclusion, these findings provide for the first time the evidence of in vitro inhibitory activities of natural polysaccharides against *C. parvum*. In vivo preliminary data showed clearly that these drugs are effective in decreasing diarrhea in ruminants.

## 030 External Quality assessment Program for *Cryptosporidium* spp. Diagnosis: experience of The French Anofel *Cryptosporidium* National Network

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**Background and aim:** The French *Cryptosporidium* National Network (FCNN) was set up in 2004 upon request of public health authorities to provide information on the incidence and epidemiology of human cryptosporidiosis in France. To evaluate the performances of the methods used in routine clinical diagnosis and in identification of *Cryptosporidium* species, the FCNN developed an external quality program dedicated to the diagnosis of cryptosporidiosis. In the present report, the results of external quality assessments (EQA) over the 2004-2012 period are provided. Methods: Four sets of EQA consisting in 3 to 5 clinical faecal samples containing, or not, *Cryptosporidium* oocysts were sent to 26 to 33 French laboratories members of the FCNN over the period. For microscopic detection, participants were asked to report results as 1/ positive/negative and 2/ number of *Cryptosporidium* spp. oocysts/2µl faecal sample. For molecular diagnosis, participants were asked to give results as positive/negative and species identification.

**Results:** For microscopic detection of *Cryptosporidium* oocysts in samples, Henriksen-Pohlenz and Heine stainings were used by 25 and one laboratory(ies), respectively. Five to 9 laboratories used original home made PCR protocols in addition to microscopy. Correct microscopic diagnosis for negative controls ranged from 87 to 96%. For samples containing > 29 *Cryptosporidium* oocysts/2µl, a correct microscopic diagnosis was given by >90% of laboratories. For samples containing *C. parvum*, *C. hominis* and *C. meleagridis* for the 2 to 4 laboratories that performed this identification. .

**Conclusion:** *Cryptosporidium* Henriksen-Pohlenz staining was the most used method for *Cryptosporidium* spp. assessment in clinical faeces samples. An improvement of the accuracy of *Cryptosporidium* detection by this method was observed for low parasitic load samples from 2004 to 2012. Overall, the results obtained of the EQA performed over the 2004-2012 period showed that diagnosis performances are similar with either microscopic or molecular diagnostic methods. Further studies aiming at defining consensual methods for molecular diagnosis are still needed.



## O31 Evaluation of *Cryptosporidium parvum* infectivity by Electrochemical Impedance Spectroscopy

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*Cryptosporidium* is an Apicomplexan genus with many species that are pathogenic to humans and animals. *Cryptosporidium* species are the etiologic agents of cryptosporidiosis, a severe diarrheal disease which can be deadly for immunodeficient people such as AIDS patients. The infectious *Cryptosporidium* oocyst is frequently transmitted via contaminated drinking water. Many techniques were developed to detect and quantify oocysts infectivity but these methods are usually time consuming, expensive and tedious. In this context, development of MicroElectroMechanical Systems applied to biology (BioMEMS) was studied to provide an automated alternative to the usual detection methods.

To this end, HCT-8 cells (human ileocecal adenocarcinoma) were cultured on interdigitated microelectrode arrays prior to being exposed to various concentrations of viable *C. parvum* oocysts. As a negative control, the cell culture was exposed to heat-shocks inactivated oocysts. Cell response to infection was continuously monitored for 60h by impedance spectroscopy with a frequency range of 100 Hz to 1 Mhz. Results showed a dose dependent response between the percentage of viability of oocysts and the impedance magnitude at 30 kHz. Furthermore in contrast with inactivated oocysts, infectious samples triggered a cyclic increase of impedance with a 9 hours period.

Immunofluorescence microscopy suggests that these impedance magnitude increases are correlated to an accumulation of parasitic single forms such as sporozoites, trophozoites and merozoites and could thus provide insights in the parasite's biological cycles. In conclusion, our data present for the first time a strategy based on impedimetric properties to assess infectivity of oocysts on cell cultures and shine new light on the parasite itself. This method, coupled with microfluidics, could also be used to screen anti-*Cryptosporidium* therapeutic drugs.

**O32**      **A novel method for simple and sensitive detection of *Cryptosporidium parvum* by surfactant extraction treatment (SET) and quantitative reverse transcription-PCR**

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*Cryptosporidium* is an intestinal protozoan parasite and causes waterborne gastrointestinal disease worldwide. The *Cryptosporidium* oocyst has a robust wall, which is resistant to many environmental factors as well as to normal water disinfection processes. Therefore, detection of the oocyst in raw water sources is considered important for drinking water quality control. Successful extraction and detection of nucleic acid from the robust oocyst usually requires freeze-thaw cycling, enzyme and surfactant treatments, and nucleic acid purification. As an alternative to these complicated procedures, we have established surfactant extraction treatment (SET) that can extract DNA from the oocyst using only the anionic surfactant sodium dodecyl sulfate and the nonionic surfactant Tween 20. The DNA extracted by SET can be amplified by PCR without nucleic acid purification. The amount of target rRNA is known to be much larger than that of target rDNA. Accordingly, this study discussed the simple and sensitive detection of the 18S rRNA gene from *C. parvum* oocyst by SET and quantitative reverse transcription-PCR (RT-qPCR). As a result, the detection of 10-3 oocysts per test tube was successful by SET and RT-qPCR, without nucleic acid purification. This data also proves that SET can be applied to not only Taq DNA polymerase but also AMV reverse transcriptase. This work was supported by JSPS KAKENHI Grant Number 25420559 and the Kurita Water and Environment Foundation.

## HOST-PARASITE INTERACTIONS AND PATHOLOGY

**O33**      **Host-parasite interactions in giardiasis: From effects on microbiota to immuno-modulation**

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The pathophysiological mechanisms responsible for acute and chronic disease upon infection with *Giardia duodenalis* remain incompletely understood. Acute *Giardia* infections have been associated with decreased intestinal inflammatory responses via mechanisms that remain obscure, and enteric infections may initiate post-infectious intestinal disorders (eg. irritable bowel syndrome [IBS]) via unknown mechanisms. The *Giardia* genome contains at least 20 cathepsin-like cysteine proteases, most of which have no known function. Much of our understanding of the interaction between enteric pathogens and the host-protective resident microbiota has come from studies focusing on populations collected from fecal samples, and therefore not entirely representative of the adherent mucosal microbiota biofilms. In an attempt to determine whether modulation of the resident intestinal microbiota may arise from acute exposure to *Giardia*, our studies investigate the effects of *G. duodenalis* and multispecies bacterial biofilms cultured from healthy human intestinal mucosal biopsies. Together, the research presented here sheds new light on how *Giardia* cathepsins may directly inhibit acute infiltration by neutrophils, and on how this parasite directly modifies the human intestinal microbiota biofilm structure and composition, with a significant increase in bacteria belonging to the class *Clostridiales*. The findings also demonstrate for the first time how acute infection with *Giardia* causes intestinal hypersensitivity in a model of neonatal rats, in the small as well as in the large intestine. *Giardia* cathepsin B proteases are able to cleave IL-1 $\beta$  or *Salmonella*-induced pro-inflammatory CXCL-8 and block neutrophil chemotaxis, which may contribute to the absence of overt inflammatory cell infiltrates in acute giardiasis, and explain in part why in developing countries, children infected with *Giardia* may present with less inflammatory diarrheal disease. These cathepsins are also implicated in the *Giardia*-induced alterations of the mucosal microbiota. In germ-free mice, dysbiotic microbiota on their own (i.e. normal microbiota after exposure to *Giardia*) significantly increase the numbers and size of lymphocyte aggregates in the intestinal mucosa, and augment tissue mast cell tryptase and eotaxin production. Microbiota rendered dysbiotic by exposure to *Giardia* are able, also on their own, to significantly increase tissue concentrations in human intestinal biopsy tissues of numerous pro-inflammatory mediators (IL-1, chemokines, RANTES etc). In conclusion, *G. duodenalis* modifies human intestinal microbiota biofilms and increases numbers of pathogenic planktonic bacteria in these biofilms. We speculate that microbiota dysbiosis caused by giardiasis may contribute to the development of post-infectious intestinal inflammatory disorders like IBS, long after the parasite has been eliminated.

## O34 Determinants of *Cryptosporidium* and *Giardia* virulence

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We have sought after species specific genes that might function in determining host specificity and serve as gold standards for species/Assemblage Determination and markers for virulent strains. We utilized comparative genomic and proteomic screens to identify potential genes which were specific to either human infective *Cryptosporidium* species or *Giardia* assemblages. We individually validated genes and proteins displaying specificity in the laboratory using a range of hybridisation and protein detection assays. Our work highlights novel contingency loci encoding secreted proteins in *Giardia* and *Cryptosporidium* as probable virulence factors which are also able useful targets for strain discrimination and risk assessment.

**O35**      **Representational difference analysis (RDA) identifies up-regulated genes in the murine intestinal epithelial cell line (IEC-6) in response to *Giardia duodenalis* infection**

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*Giardia duodenalis* is an enteric pathogen that causes diarrhoea in humans. So far, the molecular events underpinning cell responses to *Giardia* infection are unclear. Therefore, we used representational difference analysis (RDA) to identify gene transcriptional responses in intestinal epithelial cell monolayers (IEC-6), each incubated with a *G. duodenalis* isolate (WB, P-1, NF, and GS/M) for 2 h and 6 h. RDA showed that all four isolates modulated many cellular processes in IEC6 cell monolayers (e.g. transcription, translation, metabolism, signalling, vesicular trafficking). At 2 h, the infected IEC-6 cells exhibited a transcriptional response associated with DNA repair, the attenuation of oxidative stress, suppression of apoptosis, and repair of tissue and extracellular matrix thus leading to the promotion of cell survival. At 6 h, IEC-6 cells transcribed a wide range of genes associated with hypoxia, oxidative stress, and the induction of apoptosis. The up-regulation of the genes associated with immune suppression, ubiquitin-like FUBI (fau) and transforming growth factor beta (tgfb1), was detected and might account for the absence of inflammation in giardiasis. A wider range of genes associated with tissue repair and extracellular matrix was transcribed in IEC-6 cells infected with the GS/M isolate as this isolate is highly adhesive to cells and might have induced more cellular damage as a result of attachment. This study shows that disease mechanisms in giardiasis are rather multifactorial involving trophozoite attachment, oxidative stress, hypoxia, nutrient depletion specifically glucose, and apoptosis.

036

## Analysis of the damage in epithelial cells exposed to surface proteins, metabolic and proteolytic enzymes secreted by *Giardia duodenalis* trophozoites

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The interaction of *G. duodenalis* trophozoites with epithelial cells is a key process that allows parasite colonization and the onset of the variable symptoms and signs associated to giardial infections. Pathogenesis in giardiasis involves trophozoite attachment to epithelial microvilli concomitant to sensing responses to host microenvironmental factors that induce the release of several parasite molecules, such as surface and metabolic proteins as well as proteases.

To study the function of several *G. duodenalis* proteins various strategies were taken, which included purification of a cathepsin B-like protease (named giardipain-1), obtaining an antibody against a recombinant metabolic, secreted enzyme (enolase) and its purification and finally the transfection of *Giardia* trophozoites with the variable surface protein VSP9B10A. Our study showed that when added to epithelial cells giardipain-1 and enolase induced blebbing and cell shrinkage that caused the separation of the cells from each other and from their substrate. In addition, the purified giardipain-1 protease added to MDCK cells degraded tight and adherence junctions proteins as determined by Western blot analysis, it also decreased transepithelial resistance and induced apoptosis as determined by caspase 3 activation, PARP degradation and annexin V staining. Interestingly enolase did not cause significant caspase-3 activation upon interaction with epithelial cells. When trophozoites transfected with VSP9B10A were incubated with epithelial cells, damage to these cells was observed and changes in the localization of F-actin and tight junction proteins were detected. All together these results suggest that attachment of *G. duodenalis* trophozoites to epithelial cells is a multifactorial process that includes release of parasite components that damage epithelial cells and can therefore be considered as virulence factors.

**O37**      **Measuring the effects of *Giardia duodenalis* on transcellular and paracellular transport in enterocytes**

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Intestinal enterocytes are responsible for absorbing nutrients and water. In addition, this single layer of cells is perhaps the body's most important barrier between potential external toxic compounds and harmful pathogens. *Giardia duodenalis* infects the small intestines of different animals and affects normal cell function of intestinal epithelial cells in various ways. Reduced micro villous height, rearrangement of tight junction protein complexes, and increased expression and mobilization of sodium glucose transporters (SGLT-1) are examples of effects shown to be induced, in vitro and in vivo, by *Giardia*. Nevertheless, information about how *G. duodenalis* interferes with normal cell functions of enterocytes is relatively scarce, and we still do not have the full picture regarding how and why the parasite causes the symptoms observed in acute and chronic giardiasis. The aim of this study was to investigate how sonicated whole cell products from *G. duodenalis* trophozoites may affect intestinal transportation using both transcellular and paracellular probes in an Ussing Chamber experimental model. This presentation will describe the establishment of an ELISAbased method to measure transport of horse radish peroxidase (transcellular probe) and creatinine (paracellular probe) and the effects of exposure of cells to different *G. duodenalis* strains (including a novel strain isolated from a dog) on transport of both these probes. Paracellular and transcellular transport were both significantly affected by exposure to *Giardia* sonicates, and effects on paracellular transport appear to be affected by isolate.

## **O38**      **Characterization of *Giardia duodenalis* enolase and its role in the interaction with epithelial cells**

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*Giardia duodenalis* is an extracellular parasite that colonizes host small intestine. Trophozoites possess specialized structures that allow adhesion to epithelial cells which is considered a key step in the establishment and maintenance of infection. During this interaction, trophozoites release into the medium non metabolic proteins and metabolic proteins. Among the latter are enzymes of the glycolytic pathway such as OCT, ADI and enolase. Due to the various functions reported for enolase, in this work we report our findings in the characterization of *Giardia* enolase (Gd-eno) and its effect on epithelial cells. For this purpose Gd-eno was cloned, expressed and purified using the pET100-directional cloning system. The purified recombinant protein (rGd-eno) showed catalytic activity as determined by enzymatic assays. A polyclonal antibody against rGd-eno was obtained and was used to determine a) the localization of the protein on trophozoites by IIF, b) the binding to plasminogen and plasmin activation and c) to study the attachment of trophozoites to epithelial cells. The protein was localized in small vesicles in the cytoplasm, as well as on the surface of the trophozoite. In silico analysis revealed that Gd-eno possesses important conserved residues for the interaction with human plasminogen which was later confirmed by in vitro binding assays and as a result of this binding a large amount of plasmin is produced. To evaluate the direct effect of the rGd-eno on epithelial cells, the purified protein was added to cultured IEC-6 and MDCK monolayers and its effect was observed at different times. Interestingly the addition of rGd-eno to the epithelial cells induced blebbing and cell shrinkage that increased within time, leading to cell separation as determined by scanning electron microscopy. Further studies showed that incubation of trophozoites with polyclonal antibody diminished the attachment of trophozoites to IEC-6 monolayers. All together these findings suggest that rGd-eno is a plasminogen receptor, a plasmin proactivator, plays an important role in trophozoite attachment to epithelial cells and provide for the first time evidence on the damage induced by this protein in epithelial cells.



**O39 Metabolic shift from ceramide to sphingomyelin: Is it critical for cyst viability and excystation of *Giardia*?**

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Although encystation and excystation are important events in the giardial life cycle, the molecular mechanism that regulates this process is not well understood. Earlier studies from our laboratory indicate that sphingolipids (SLs) play a critical role in encystation. Only five SL genes are present in this parasite, and they are transcribed differentially during encystation. While giardial serinepalmitoyltransferase (gSPT) regulates ceramide endocytosis, glucosylceramide synthase (GS) is involved in the biogenesis of encystation-specific vesicles (ESVs), cyst formation, and the maintenance of overall lipid homeostasis. To better understand the role of SL molecules and their possible metabolic transformation during differentiation, we carried out sphingolipidomic analysis by mass spectrometry, and then we compared the SL profiles in trophozoites, encysting cells, cysts, and media. We found that neutral SLs, including ceramide, hexosylceramide, dihexosylceramide, and trihexosylceramide, increased significantly, from 4- to 10-fold during encystation. Interestingly, the level of sphingomyelin (SM) became dramatically elevated (~60-fold) in cysts, although it was not present in the media, which suggests that new SMs are synthesized from ceramide either de novo or through remodeling reactions. We performed sucrose-gradient centrifugation to separate viable from non-viable cysts, and this was followed by SL analysis and in vitro excystation. We observed that there are correlations among SM level, cyst viability, and excystation. Altogether, our results indicate that a metabolic shift from ceramide to SM occurs during encystation and that this could be important for maintaining cyst viability and inducing excystation.

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## O40 Interaction between *Cryptosporidium parvum* and the intestinal microbiota

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The course of an infection is typically assumed to be determined by pathogen virulence and host immune response. New insights into the pathogenesis of enteric infections have revealed the importance of the gut microbiota in modulating the virulence of pathogens. Prompted by these observations, we undertook experiments in mice to assess the impact of *Cryptosporidium parvum* on the intestinal bacterial population and vice versa. In a first series of experiments, immunosuppressed adult mice were infected with *Cryptosporidium parvum*. DNA was extracted from daily fecal samples excreted by infected and control animals. To examine whether cryptosporidiosis affected the composition of the intestinal bacterial population, PCR products amplified from a variable region of the bacterial 16S rRNA gene were deep-sequenced using Illumina technology. Curated sequence reads from infected and control animals were compared using a measure of phylogenetic distance. In three replicate experiments, we found a small, but significant, difference in the composition of infected and control mice, suggesting that in this model cryptosporidiosis perturbs the intestinal ecology. To assess whether a reverse effect of the microbiota on *C. parvum* proliferation could be detected, in a second series of experimental infections, *C. parvum* infected mice were orally treated with antibiotics to alter the composition of resident gut microbiota. Parasite proliferation was estimated by quantifying fecal oocyst concentration on acid-fast stained fecal smears. The results showed that in average the number of oocysts per gram feces was lower in mice treated with bacitracin as compared to untreated mice. Streptomycin showed a similar effect. However, in contrast to bacitracin, streptomycin was also inhibitory to *C. parvum* proliferation in cell culture, suggesting that this antibiotic, as opposed to bacitracin, may inhibit the parasite directly. Together, these observations open new possibilities for understanding the importance of intestinal microorganisms in modulating the virulence of cryptosporidiosis and other enteric infections. In light of the continued lack of effective anti-cryptosporidial drugs, a better understanding of pathogen-microbiota interaction may provide alternative methods for managing cryptosporidiosis in humans and livestock.

# Oral Presentations

## O41      Activation of pathological immunity in giardiasis

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Infection with *G. duodenalis* can lead to defects in nutrient absorption, reduced epithelial barrier function, diarrhea, cramps, and physical and developmental delays. However, many infected individuals exhibit no symptoms at all. We have used murine infections with *G. duodenalis* in order to understand pathophysiological mechanisms of disease. Comparing infections with the Assemblage A strain WB and the Assemblage B strain GS showed that while GS infected mice exhibited reduced levels of disaccharidase activity, WB infected mice did not. Moreover, immunodeficient mice infected with strain GS did not exhibit reduced disaccharidase levels, despite having significantly elevated parasite burdens in the small intestine. CD8+ T cells are implicated in mediating disaccharidase deficiencies and we have therefore characterized the response of CD8+ T cells throughout the mucosal immune system following infection. CD8+ T cells in the lamina propria are activated following infection and express both IFN- $\gamma$  and TNF- $\alpha$ . Activation of CD8+ T cells by extracellular pathogens is unusual and we have therefore investigated mechanisms involved in initiating this response. Activation of these cells was reduced by treating mice with antibiotics, indicating a role for microbiota. Interestingly, antibiotics do not appear to reduce activation of CD4+ T cell responses. Antibiotics also reduced the amount of disaccharidase deficiency observed, consistent with the role of CD8+ T cells in this phenotype. Preliminary experiments using transgenic T cells specific for non-parasite antigens suggest that T cell activation is occurring in an antigenspecific manner. We have also explored the impact of antibiotics on the initiation of immune responses to *Giardia*. Antibiotics reduce expression of macrophage activation markers following infection and we are investigating the link between activation of innate responses by microbes and the generation of parasite-specific adaptive responses. Together our data support a paradigm in which interactions among parasites, commensal bacteria and host immune responses combine to determine the outcome of infection.

**O42 PPAR $\alpha$  and IL-17A responses associated with the intestinal immune response against the protozoan parasite *Giardia muris*.**

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The protozoan parasite *Giardia duodenalis* (*lamblia*) is one of the most commonly found intestinal pathogens in mammals, including humans. Recent research in cattle revealed the activation of the peroxisome proliferator-activated receptors  $\alpha$  and  $\gamma$  as part of the intestinal response against this parasite. The activation of PPARs can exert an anti-inflammatory effect by transrepressing the activity of several transcription factors, such as nuclear factor- $\kappa$ B (NF- $\kappa$ B) and activator protein 1 (AP1). The aim of the current study was to further analyze the role of these receptors in the host-parasite interaction and their possible impact on the development of protective immunity using a *Giardia muris*-mouse infection model. Analysis of the intestinal response in C57BL/6 mice indicated the activation of PPAR $\alpha$  in the enterocytes soon after the initial contact with this parasite, characterized by the transcriptional upregulation of PPAR $\alpha$  itself and several classic downstream target genes such as PLTP and CPT-1. In contrast to cattle, no PPAR $\gamma$  activation was observed in mice and the PPAR $\alpha$  response disappeared 1 to 2 weeks post infection, followed by a strong Th17 response with a high upregulation of IL-17A in the mucosa, peaking at week 3 post infection. The importance of IL17A in orchestrating the protective immune response was unequivocally demonstrated in an infection trial using IL17 receptor A KO mice. Whereas in wild type mice cyst secretion dropped significantly after 3 weeks of infection, the IL17RA-KO mice were unable to clear the infection. The regulation of the PPAR $\alpha$  response and its impact on the protective IL-17A response is currently under further investigation.

## O43 Flow cytometric method for characterization of human T cell responses against *Giardia lamblia*

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**Background:** *Giardia lamblia* is an intestinal protozoa, which causes infection in humans worldwide. The impact of the infection varies from asymptomatic carriers to severe disease such as malabsorption syndrome. Acquired immunity in individuals, who previously have suffered from giardiasis, has been suggested by earlier studies. Data regarding cytokine expression in T cells from *Giardia* infected individuals are limited. Specific immune responses against *Giardia* can be investigated by measuring cytokine expression and combining this data with cell proliferation and surface markers on T cells.

**Methods:** Cultures of peripheral blood mononuclear cells from one group of individuals with recent giardiasis are stimulated with *Giardia* Assemblage A and B soluble antigens, and compared to a group of low risk healthy controls. The first assay, where the cells have been stimulated for 20 hours examines the cytokine expression of TNF- $\alpha$ , IFN- $\gamma$ , IL-17a and IL-4 in addition to surface markers for central and effector memory T cells. The other assay examines proliferation by CellTrace dye dilution and activation markers HLA-DR, CD45RO, CD25 and CD26 after antigen stimulation for 6 days. Cells are then analyzed with a flow cytometer.

**Preliminary results:** CellTrace dye dilution, which is a sensitive proliferation method, shows that individuals with recent symptomatic giardiasis have increased proliferation responses against both Assemblage A and B soluble antigens. Smaller non-specific responses are found in low risk healthy controls. TNF- $\alpha$  expression varies in-between individuals, but is generally higher in individuals with recent giardiasis. IL-4 and IL-17a responses are low in both of the groups. The individuals with recent giardiasis have higher percentages of activated T cells compared to the low risk healthy controls. Data collection is ongoing and further analysis will be presented at the conference.

**Conclusion:** These assays combined can give detailed insight in human cellular immunity against *Giardia*, by both acquiring early cytokine profiles of effector memory T cells and later T cell proliferation and activation responses.

# Oral Presentations

## O44 Cryptosporidiosis induces caspase-1 driven cytokine cascade

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Nod-like receptors (NLRs) are important in the early host cell infection, leading to inflammasome formation and caspase-1 activation. Production of active IL-1 $\beta$  and IL-18 is generated through cleavage by caspase-1. The latter cytokine is an important inducer of IFN- $\gamma$  in the protection of *Cryptosporidium*. In order to determine the importance of caspase-1 in host susceptibility we challenged adult caspase-1 knockout mice with  $1 \times 10^6$  oocysts. We found that infection was significantly increased (10 fold) in adult caspase-1 knockout compared to wild type mice. We also infected epithelial cells in vitro (HCT-8 and CaCo2) and found that expression of caspase-1, IL-1 $\beta$ , IL-18 and the nod-like receptor NLRP3 genes were upregulated in response to infection. Treatment of infected HCT-8 cells with nigericin (an inflammasome inducer) significantly reduce infection (>95% at 3 micromolar). Caspase-1 and IL-1 $\beta$  are up-regulated in human MoDCs in response to incubation with live sporozoites. Lastly, increased secretion of IL-18 and IL-1 $\beta$  by bone marrow dendritic cells (BMDCs) was observed in response to *C. parvum* following incubation with cryptosporidial antigens. These data support a role for inflammasomes in recognition of *Cryptosporidium* immunity.

## O45 Can infection with one genotype of *C. parvum* protect against infection with another?

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Currently there is little knowledge about the development of immunity to *Cryptosporidium*, in humans it is possible to become infected with *Cryptosporidium* in childhood and in adulthood. In livestock however, diarrhoea caused by the parasite is generally only seen in neonatal livestock and not older animals. It is not known if infection with one genotype of *C. parvum* will provide protection against infection with another. It is possible that if one genotype does provide protection against another then there is potential for the development of control measures using a less virulent isolate to protect against infection from severe disease caused by a more virulent genotype.

There are few studies which have looked at the effect of sequential infection of *C. parvum* on neonatal livestock. Two studies, one in sheep [1] and one in cattle [2] have looked at the effect of age on infection with *Cryptosporidium* species. Both studies showed that animals exposed to the parasite at a younger age do not have such severe disease when exposed a second time showing that the animals were able to develop protective immunity to a homologous challenge. Most of the animal studies investigating immune responses to infection were carried out before the discovery of many of the species and genotypes which are now known to infect farm livestock.

An experiment challenge was carried out to test the development of resistance to homologous and heterologous *Cryptosporidium* infections in lambs using two distinct *C. parvum* isolates. Thirty-six neonatal lambs were split into 6 groups: two to test age-related susceptibility, two homologous and two heterologous challenge groups. The lambs were kept for 8 weeks until oocyst shedding stopped, total faecal output and weekly blood samples were collected from each lamb. Clinical data was recorded daily. Oocyst counts are used to determine the shedding profile of each group with PCR to confirm the genotype being shed. Antibody levels will be compared with shedding data to confirm infection status.

Currently we have preliminary results showing that animals infected for the first time later in life exhibit few, if any, clinical signs of disease, although these lambs can still shed large numbers of oocysts. We also found evidence that some isolates can cause slightly more severe and prolonged diarrhoea than others. Evidence of protection against homologous and heterologous challenge will be discussed.

### References:

[1] Ortega-Mora LM, Wright SE. Age-related resistance in ovine cryptosporidiosis: patterns of infection and humoral immune response. *Infect Immun.* 1994 Nov; 62(11):5003-9.

[2] Harp JA, Woodmansee DB, Moon HW. Resistance of calves to *Cryptosporidium parvum*: effects of age and previous exposure. *Infect Immun.* 1990 Jul; 58(7):2237-40.

## O46 DNA damage repair in *Giardia duodenalis*: characterization of putative rad52 gene.

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Genetic recombination is a central process in several pathways in most eukaryotic organisms. In *Giardia duodenalis*, however, DNA damage repair has not been fully characterized. Our group has characterized some proteins involved in DNA repair. During the process of homologous recombination, the main DNA repair pathway involves the participation of Rad52 that mediates the incorporation of rad51, the eviction of RPA and is also in charge of mediating second strand annealing. This work aimed to characterize the biochemical functions of the putative Rad52 protein of *Giardia duodenalis*. For this purpose the protein was expressed in a heterologous bacterial system and , purified for further analysis which included in vitro DNA binding, ssDNA annealing, ability to form heptameric structures by electromicroscopy. Also this protein was detected in wild type trophozoites as well as in parasites exposed to ionizing radiation to detect DNA damage repair. Our results show that *Giardia duodenalis* Rad52 bound preferentially to single DNA strand; also it accomplished efficiently annealing of complementary DNA even in the presence of GdRPA1. GdRPA1 normally inhibits this alignment due to its affinity to DNA. Rad52 forms structures like rings which allow efficient binding to DNA. Interestingly, RAD52 protein levels increased in the parasite in response to DNA damage when exposed to a dose of 100 Gy. All together these results suggest that the putative sequence of Rad52 in *Giardia duodenalis* is a bona fide Rad52 protein which is expressed and participates in DNA repair in this parasite.



## O47 Electron microscopic study of in vitro axenic development of *Cryptosporidium parvum*

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The in vitro development of *Cryptosporidium parvum* in an axenic (cell-free) culture system was studied by transmission electron microscopy (TEM). The purified oocysts were subjected to bleaching and excystation process before culturing in the axenic culture media. Using the TEM, we detected early *C. parvum* trophozoites, ovoid in shape, 1, 06 x 1, 07 µm, containing a large nucleolus filling most of the nucleus and a Golgi complex next to the nucleus. Dividing and mature meronts containing six to eight fully formed merozoites (Meront I) and four formed merozoites (Meront II), 4.9 x 4.7 and 2.6 x 3 µm respectively, were detected attached to a small residual body within as well as outside of the oocysts in the medium within the first 24 hours. Sexual stages including the macrogamonts and microgamonts were observed within 3 days of culturing. The macrogamonts were spherical to ovoid measuring 1.2 (1-1.3) x 1.5 (1.13-1.87) µm. They had two distinct types of wall-forming bodies (WF) WF1 and WF2 seen at the periphery as well as amylopectin granules. The microgamonts were 1.6 x 1.5 µm in size with a nucleus showing lobe projection, condensation of chromatin and peripherally arranged budding microgametes. Within each mature microgamete there was a large area of granular substance containing groups of microtubules surrounding the electrondense nucleus. The development of new thin and thick-walled oocysts was noticed within 7 days of culturing. New oocysts undergoing sporogony were also seen. They had a large oocyst residuum, polysaccharide granules, and remnants of wall forming bodies. The thin-wall new oocysts were spheroid to ellipsoid in shape, measuring 3.7 x 3.8 µm. The new developed thick-wall oocysts measuring 3.5 x 3.7 µm, were also observed having 4 layered walls; an outer layer with irregular knobs, followed by an electron-transparent space, underneath which is the third thick inner layer and then the fourth and innermost layer. Underlying this thick four layered wall, there is a double membrane that surrounds the developing oocyst containing vacuolation and indentation of the inner mass zone. This study has led to further novel findings as visualisation of the different *Cryptosporidium* stages developing within the shells of the oocysts, as well as detection of gregarine-like stages and syzygy. We have also noticed that the outer membrane of some of the life-cycle stages extends as a parasitophorous vacuole-like membrane. The present study confirms the axenic in vitro development of *C. parvum* stages and for the first time describes their ultra-structure by TEM. It is an indication that the *Cryptosporidium* can develop outside its host. Further studies are necessary to optimize the system towards development of an in vitro model for mass cultivation of the different life cycle stages of *Cryptosporidium* species to facilitate its use for future approaches of developmental biology of the parasite.

## MOLECULAR EPIDEMIOLOGY AND TYPING

O48

### Epidemiology and molecular genotyping of *Cryptosporidium* and *Giardia* in cattle, buffalo and humans in the Ismailia province of Egypt

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*Cryptosporidium* and *Giardia* are considered two of the most common protozoan parasites transmitted via drinking water or animal-to-human and affecting the small intestine of animals and humans causing diarrhoea. Faecal samples were collected from cattle and buffalo (n=804, 191 herds) of smallholder farms and diarrhoeic children (<10 years, n=165) in the Ismailia province. All samples were first screened by the immunochromatic RIDA®QUICK test. Positive samples and 10% of randomly selected negative animal samples were further tested by polymerase chain reaction (PCR) assays aimed at the partial amplification of the 18S ribosomal DNA gene and 60 kDa glycoprotein (GP60) encoding gene of *Cryptosporidium*. For *Giardia*, SSU rDNA-specific real time PCR was used and positive samples were genotyped based on the triosephosphate isomerase (tpi), glutamate dehydrogenase (gdh) and  $\beta$ -giardin (bg) genes. All human samples were analyzed and genotyped by RIDA®QUICK test and PCR. Prevalences of *Cryptosporidium* and *G. duodenalis* based on RIDA®QUICK were 19.5% and 7% in animals and 6.7 and 10% in children. Respective estimated prevalences based on PCR were 32.2% and 53% in animals and 49% and 21% in humans. For *Cryptosporidium*, subtype family IId (mostly genotype IIdA20G1) predominated over IIa (genotype IIaA15G1R1) in animals, while subtype families IIa (genotypes IIaA15G1R1 and IIaA15G2R1) and IId (genotype IIdA20G1 only) were identified in equal amounts in humans. For *Giardia*, genotyping revealed a predominance of Assemblage E-type (82%), followed by A-type (16%) sequences in animals. In children, Assemblage B-type (68%) was predominant, followed by AII-type (32%) sequences. *Giardia-Cryptosporidium* spp. co-infections were observed in 36% of the animals and 11% of the children. Assuming that co-infection will happen by chance, its prevalence can be estimated by the product of the prevalences of individual infections. Accordingly, prevalences of co-infection can be expected in 17% of animal and 10% of human infections. In conclusion, the Ismailia province of Egypt is highly endemic for *Cryptosporidium* and *Giardia* infections suggesting a widespread transmission scenario. Drinking tap water may increase the risk of contracting the infection with both protozoans. In *Cryptosporidium*, zoonotic transmission due to close contact with animals was a statistically significant risk factor of human infections, but animal contact appears to be negligible in *Giardia*.

**O49 Molecular typing of Swedish *Cryptosporidium* isolates during 2013 – few domestic cases caused by anthroponotic species/subtypes**

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In 2010 and 2011 two large waterborne outbreaks of *Cryptosporidium hominis* infection in which 47,000 persons were affected occurred in Sweden. These incidences in combination with several previous foodborne outbreaks highlighted a need for better surveillance of this parasite in Sweden and a national typing project was initiated.

Local microbiology laboratories were requested to send in fecal samples from all diagnosed human *Cryptosporidium* cases to the Swedish Institute for Communicable Disease Control (SMI) for species differentiation and subtyping. Isolates from 116 patients, representing about half of the cases reported to the national surveillance system (SmiNet) during 2013 were successfully genotyped. Most of the patients (n=69) had acquired their infection in Sweden. Of these *C. parvum* was the dominant species (n=61), and only one domestic *C. hominis* case was identified. Furthermore, seven domestic cases caused by more uncommon species/genotypes were detected: *C. ubiquitum* (n=2), *Cryptosporidium* chipmunk genotype I (n=1), *C. felis* (n=2) as well as two hitherto unidentified genotypes. Interestingly one of the *C. felis* cases was caused by confirmed zoonotic transmission.

Among patients infected abroad (n=44) *C. parvum* was the most common species found (n=24), followed by *C. hominis* (n=15). In addition *C. hominis* monkey genotypes (n=2), *C. meleagridis* (n=2) and *C. viatorum* (n=1) were identified. No epidemiological data was obtained for three *C. parvum* infected patients. All *C. parvum*, *C. hominis* and *C. meleagridis* isolates were successfully subtyped at the GP60 locus. Five *C. parvum* (IIa, IIc, IId, IIIn, IIp), five *C. hominis* (Ia, Ib, Id, If, Ij) and two *C. meleagridis* (IIIb and IIIe) allele families were identified. Two domestic outbreaks were investigated during the study period: one foodborne and one among veterinary students.

This project has given us further insight into which species and subtypes are causing cryptosporidiosis in Sweden. With the large 2010–2011 waterborne outbreaks caused by *C. hominis* IbA10G2 in mind, we were surprised not to find any domestic cases of infection with this subtype during 2013. Surprisingly, we found that the percentage of patients infected in Sweden with uncommon species/genotypes (10%) was much higher than expected based on data from other studies.

050

## ***Cryptosporidium* increase in the summer of 2012 in the Netherlands analysed by GP60 genotyping.**

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In the Netherlands the number of human cases with cryptosporidiosis increases at the end of the summer, a well known phenomenon, noticed in other countries as well. In the summer of 2012, however, the number of cryptosporidiosis cases was extraordinary high. This rise of cryptosporidiosis cases led to an epidemiological investigation into the cause and to a large genotyping effort on *Cryptosporidium* in patients by the National Institute of Health (RIVM). The epidemiological study, published by Fournet et al [1], did not reveal a clear common cause. The unusual rise was seen in various parts of The Netherlands. Data from England, Wales and Germany also revealed a higher seasonal summer peak than expected from previous years. We received approximately 500 DNA or fecal samples from microbiological labs from all over The Netherlands. Genotyping, using PCR on GP60 and subsequent sequencing was successfully on half of those samples. As could be expected from previous years *C. hominis* was the dominant species. This season it was found in 90% of all genotyped samples, higher than expected, 82% being genotype IbA10G2. The second in line is genotype IaA14 (8%) and the third is IIaA15G2R1 (4%). In total 15 different genotypes were detected, mostly in only one or two samples. A selection of the IbA10G2 samples from 2012 and some IbA10G2 samples from previous years were analysed with 7 microsatellite markers in order to detect genetic differences within this genotype. Complete or partial genotypes were established in 16 samples from 2012 and 28 from previous years. No differences were found. This might indicate that the IbA10G2 genotype is homogeneous genetically but more indepth analyses are being performed. So far, the genotyping analyses do not further elucidate the cause of the rise of cryptosporidiosis in 2012.

### Reference:

[1] Fournet et al. Simultaneous increase of *Cryptosporidium* infections in the Netherlands, the United Kingdom and Germany in late summer season, 2012. Euro Surveill 2013

**051**      **Comparative genomics of *Cryptosporidium hominis*: genetic recombination as a driving force for emergence of virulent subtypes**

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*Cryptosporidium hominis* is the dominant species responsible for human cryptosporidiosis. Within the species, one subtype at the gp60 locus, IbA10G2, is the most virulent parasite, responsible for all *C. hominis*-associated outbreaks in Europe and Australia, and is the dominant outbreak subtype in the United States. In recent years, a newly emerged subtype, IaA28R4, is becoming a dominant *C. hominis* subtype in both sporadic cases and outbreaks. As the genome of only one *C. hominis* isolate, TU502 of the IaA25R3 subtype, has been sequenced thus far, the mechanism for the hypertransmissibility and virulence of IbA10G2 and IaA28R4 is not clear. In this study, we sequenced the genome of two isolates each of the two subtypes from three waterborne outbreaks and one sporadic case in the United States by 454 and Illumina technologies. Altogether, 8.61-9.05 Mb of *Cryptosporidium* sequences in 45-831 assembled contigs were obtained from these isolates, representing 94.35-99.47% coverage of the expected genome. The genome of these isolates had 96.90-97.01% and 99.78-99.83% nucleotide sequence identity to the published genomes of *C. parvum* and *C. hominis*, respectively. They also had an almost complete syntany in gene organization to the two previously sequenced species; all potential gene re-arrangements were seen around physical gaps in the published *C. parvum* genome, probably as a results of misassembly of scaffolds in the latter. Nevertheless, several major insertions and deletions were seen between *C. hominis* and *C. parvum* genomes, including two coding regions. The four *C. hominis* genomes were almost identical to each other and were divergent in nucleotide sequences from the reference IaA25R3 genome at most highly polymorphic regions examined. The only major sequence differences among the four isolates sequenced in this study were in the 5' and 3' ends and gp60 region of chromosome 6, largely the result of genetic recombination. The sequence similarity among isolates of the two dominant outbreak subtypes and genetic recombination in chromosome 6, especially around the putative virulence determinant gp60 region, suggest that genetic recombination plays a major role in the emergence of hyper-transmissible *C. hominis* subtypes, as indicated recently by our population genetic study of chromosome 6 sequences of virulent IbA10G2 subtype and other subtypes. The gene insertions and deletions could be responsible for the major difference in host-specificity between *C. hominis* and *C. parvum*. (Supported by NSFC grants 31229005 and 3110103901)

O52

## Comparison of Sanger and Next Generation sequencing for typing *Cryptosporidium* isolates

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The advent of next generation (NextGen), high-throughput sequencing platforms is rapidly revolutionizing several research areas in the Life Sciences. Sequencing of genes, genomes, and metagenomes is now routinely carried out in many laboratories; however, the application of NextGen technologies to characterize *Cryptosporidium* infections in biological samples is in its infancy. This approach may likely prove to be an ideal technique to genetically audit the species composition of multiple samples in parallel, in a rapid and cost-effective fashion. In the present study, we compared Ion Torrent and Pyrosequencing of *Cryptosporidium*-positive faecal samples at the 18S and actin loci, and compared the results to Sanger sequencing.

## O53 **CryptoTyper, a software package for automated GP60 subtyping of *Cryptosporidium***

**Erik Alm**<sup>1</sup>, Jessica Beser<sup>1</sup> and Marianne Lebbad<sup>1</sup>

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A software package for automated determination of allele families and repeat patterns in the 60 kDa glycoprotein gene (GP60) of *Cryptosporidium* spp. is in development at the Health Protection Agency of Sweden. The package performs analysis of raw Sanger read data and/or processed sequence data and outputs sequence QC data and the complete GP60 subtype name, including rare repeats. CryptoTyper will be available for download together with a bundled reference dataset for the most common species and allele families, this dataset can later be expanded with rare and novel allele families. The software uses a two-stranded repeat-counting technique if bidirectional Sangerdata is supplied, this reduces the amount of counting errors drastically and allows for accurate counting of very long repeats for subtypes such as IaA33R3.

**O54**      **Harmonisation of multi-locus subtyping schemes for public health investigations of *Cryptosporidium parvum* and *Cryptosporidium hominis***

**Rachel M Chalmers<sup>1</sup>**, Stephen J Hadfield<sup>1</sup>, Claire Alexander<sup>2</sup> and Emily Hotchkiss<sup>3</sup>

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Multilocus typing can be informative in the investigation of pathogen population structure, transmission and epidemiology, and has already improved our understanding of *Cryptosporidium parvum* and *Cryptosporidium hominis*. However, comparison of epidemiological data across studies, and between countries, has been hampered by the absence of a standardised, multilocus subtyping scheme. Importantly, sampling frames and prevailing ecological determinants of transmission also need to be taken into account in interpretation.

For public health investigations requiring a rapid response, identification of variation in the length of DNA fragments generated by amplification of microsatellite (up to 6 bp) or minisatellite (>6 bp) markers, driven by the number of tandem repeating motifs, can be measured rapidly and costeffectively by gel or capillary electrophoresis. Sequence analysis additionally identifies heterogeneity within the fragments, but during outbreak investigations multilocus fragment typing (MLFT) would provide more rapid assessment of relationship between isolates.

Service providers for neighbouring countries within the UK are working together to harmonise multilocus subtyping schemes that can be used to support public health investigations of sporadic and outbreak cases of cryptosporidiosis caused by *C. parvum* and *C. hominis*. Using the hierarchy of marker desirability identified by Robinson and Chalmers [1] and from laboratory-generated data, we first identified up to ten markers per species. Standardised, single round, PCR formats were developed for each marker. These are being reduced to eight per species following local evaluation with the least discriminatory markers being eliminated from each panel, enabling 96 well format assays to be used with ease. The markers being evaluated for *C. parvum* are: MM5, MM18, MM19, TP14, MS9, GP60, MSA, MSF and MSD. Those for *C. hominis* are: MSG, MSE, MSB, CP47, MS5, GP60, TP14 and MSC6-7.

Options for normalising fragment sizes across different analytical platforms are being explored and a library of alleles established for inter-laboratory validation. This should enable wider application of the panel for international investigations. Applications and findings will be presented at the conference.

**Reference:**

[1] Robinson and Chalmers 2012. Assessment of polymorphic genetic markers for multi-locus typing of *Cryptosporidium parvum* and *Cryptosporidium hominis* Experimental Parasitology 132: 200–215



## O55 Generation of a *Giardia duodenalis* biobank for functional epidemiology

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The pathophysiology and underlying parasite virulence factors of *Giardia duodenalis* infections are largely unknown. In addition, drug susceptibility information of filed isolates, in particular from treatment refractory patients, is scarce. The aim of the current study is to establish a biobank and data base collection of *G. duodenalis* isolates from Germany in order to create a tool for functional epidemiology.

*G. duodenalis* cysts are isolated from faecal samples and subsequently subjected in vitro to excystation and culture protocols, in order to establish trophozoite cell lines. Parasites are genotyped based on the genomic sequence of established marker genes. Trophozoite cell lines are characterized in functional assays, e. g., regarding drug susceptibility or potential virulence factors. Eventually, epidemiological and biological data will be linked within one data base. Purified cysts of 195 samples (from 319 total isolated between November 2010 to December 2013) were subjected to excystation. In 53 samples (27%), viable trophozoites were detectable after one week of culture, and long-term in vitro parasite lines could be established for 15 isolates (8%). Genotyping of 288 samples revealed *G. duodenalis* type B in 73% of the samples. In contrast, 90% of samples for which in-vitro cultures could be established belonged to type A indicating that culture conditions preferentially supported growth of Assemblage A isolates. Five of the new isolates have been characterized in more detail. Both, the growth rates and the drug susceptibility to metronidazole and orlistat, a lipase inhibitor with potent *Giardia* growth inhibitory effects in vitro, were significantly different depending on the genotype analysed. In conclusion, the establishment of 15 new *G. duodenalis* isolates enables a starting point for functional analysis. In particular, the analysis of drug susceptibility and the analysis of the diversity of potential virulence factors will be analysed in future. However, the generation of long-term in vitro cultures of *G. duodenalis* trophozoites from primary samples remains challenging. For the establishment of a functional epidemiology approach, it will be necessary to establish improved culture protocols, particularly for parasites belonging to the genotype B.

## O56 What is a *Cryptosporidium* isolate?

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The *Cryptosporidium* sporozoite represents the basic replicating unit carrying the parasite's haploid genome. Due to the lack of simple methods for axenic culture of the sporozoites, it has been consensually agreed to define the oocysts recovered from the individual hosts as operational taxonomic units of genetic studies of cryptosporidiosis. This convention implies a recent common ancestor and genetic homogeneity of the sporozoites within the host, arguably a fallacious assumption given what is known about the parasites' life cycle. In this presentation, the sources of genetic variation of the sporozoites within the hosts will be addressed and new and previous evidence supporting the model of *Cryptosporidium* 'isolates' potentially being collections of heterogeneous sporozoites, reviewed. The implications of this model on molecular source-tracking using PCR-Sanger sequencing will also be discussed.

## CLINICAL AND PUBLIC HEALTH

### 057      **Sequelae of human *Cryptosporidiosis* in patients living in Östersund and Skellefteå during two large waterborne outbreaks**

**Johan Lindh<sup>1</sup>**, Micael Widerström<sup>2</sup>, Michael Lilja<sup>3</sup> and Stephan Stenmark<sup>4</sup>

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**Background:** During the winter 2010/2011 two waterborne outbreaks of *Cryptosporidium hominis* occurred in the cities of Östersund and Skellefteå, Sweden. Approximately 47,000 humans were affected and demonstrated three or more diarrheas daily and/or watery diarrhea. To our knowledge no studies have been performed following up the long term health effects from a large *Cryptosporidium* outbreak.

**Methods:** During the outbreak questionnaires were sent to a representative part of the populations in Östersund and Skellefteå. The following case definition was used: a person living in Östersund or Skellefteå during the outbreak period that had had three or more diarrheas daily and/or watery diarrhea. In the following studies, follow-up questionnaires were sent to same groups that received the outbreak questionnaire. Questionnaires were developed for adults and children and included questions about a variety of symptoms, e.g. joint pain, pain in the eyes, fatigue, abdominal pain, diarrhea and headache. The questionnaires were sent 6 months (Östersund and Skellefteå), 12 months (Skellefteå) and 24 months (Östersund) after the outbreak. Binary logistic regression was used to detect differences between the two groups and fuzzy cluster analysis to detect patterns in the different variables of symptoms.

**Results:** Answered questionnaires 6 months after outbreak were received from 308 case patients and 479 noncase subjects. The case patients significantly reported to have more symptoms than the non-case subjects. Among the highest could loss of weight (odds ratio [OR] 3.07), loss of appetite (OR 3.06), three or more diarrheas daily (OR 3.04), watery diarrhea (OR 2.9), abdominal pain (OR 2.9), headache (OR 2.22) and pain in the eyes (OR 2.05) be seen. The variables were further analyzed for patterns and three groups were identified. These were named stomach/intestine (OR 2.27), fatigue/headache (OR 2.18) and joint pain (OR 1.87). Similar results were also demonstrated 12 months and 24 months after the outbreak and will be presented.

**Conclusions:** Infection with *C. hominis* increases the risk of getting sequelae at least 24 months after primary infection.

**O58**      **Investigation of the long term health sequelae following acute infection with *Cryptosporidium parvum*: an outbreak follow-up study**

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To investigate health sequelae (including irritable bowel syndrome, IBS) occurring after resolution of acute *C. parvum* infection in adults associated with a food-borne cryptosporidiosis outbreak in England, we undertook a longitudinal study. A total of 197 patients, resident in England, aged 16 years or over, diagnosed with *C. parvum* infection during May 2012 were asked to complete self-administered questionnaires - either using a secure online platform or paper format - at 6 and 12 months after diagnosis. Informed consent was obtained from all participants. A total of 54 participants were recruited to the study (recruitment rate=27%). Females were overrepresented among participants (74%) in comparison with potential participants (67%). There was no statistically significant difference in the mean age of those who did and did not take part ( $p=0.18$ ). 14 males and 36 females completed the 6-month follow-up questionnaire online (mean age 39.8 years). Having accessed the web-link, only 2 people failed to complete the questionnaire (completion rate 96%). A further 4 females completed the questionnaire in paper format (statistically significantly older mean age of 67.8 years). 39 of 54 recruited participants completed the 12-month follow-up questionnaire (retention rate=72%). Pre-existing IBS was reported by 11 people. The severity of acute cryptosporidiosis symptoms did not appear to be related to pre-existing irritable bowel syndrome: 55% of participants with and without IBS reported acute cryptosporidiosis symptoms as severe. At 6 months follow-up, 9 of 54 (17%) people reported symptoms which fulfil the Rome III criteria for diagnosis of IBS, 2 of whom did not report pre-existing IBS. At 12-month follow-up 44% of those with pre-existing IBS reported worsening of IBS symptoms in the 12 months after acute cryptosporidiosis.

26% of participants reported that they did not receive any information or advice about cryptosporidiosis. We conclude that a secure, online platform provides an efficient, accessible and acceptable method for personal health data collection from study participants. There is scope for improving communication between patients and professionals involved in the management of cryptosporidiosis.

The significance of reported rates of IBS and IBS-consistent symptoms requires further research, and this study has informed the design of a Wales-wide prospective investigation of post-acute health sequelae, currently underway.

## O59 Treatment with cytokine modulating agents - an emerging clinical risk factor for cryptosporidiosis

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Cytokine-modulating drugs inhibit the activity of tumour necrosis factor alpha (TNF- $\alpha$ ). Over the past few years the UK National Institute for Health and Care Excellence (NICE) has introduced recommendations for anti-TNF agent use in several inflammatory conditions, including rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, inflammatory bowel disease and ankylosing spondylitis. As a result an ever-larger group of patients in diverse specialities are now receiving this treatment, originally the preserve of rheumatologists. Even before treatment with anti-TNF agents has started, many of these patients are already immunosuppressed by other medications for their underlying disease. Predictably, the use of these drugs is complicated by a risk of reactivation of latent infections, and of new infectious episodes. For example, there is a 4-8-fold increased risk of reactivation of latent tuberculosis. Other recognised risks include reactivation of herpes zoster and hepatitis B infections, worsening of hepatitis C infection, and sepsis. In this presentation we describe a clinical case of cryptosporidiosis in a young immunosuppressed patient with inflammatory bowel disease (Crohn's disease) receiving the anti-TNF agent adalimumab, and review the literature pertaining to *Cryptosporium* infection in these patients. This relatively new patient group is expanding and represents a new risk group for cryptosporidiosis which has hitherto been little-recognised. Clinicians in a range of specialties (eg dermatologists and gastroenterologists as well as rheumatologists) need to be aware of the significance of diarrhoea in these patients, and have a low threshold for requesting testing for *Cryptosporidium* as well as other pathogens.

060

## ***Cryptosporidium* infection associated with maternal HIV among children under five years presenting with acute diarrhea in two Western Kenya Hospitals**

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Infection with *Cryptosporidium* species (spp.) has been identified as a frequent cause of diarrhea in low-resource settings. We measured prevalence of *Cryptosporidium* spp. in children with acute diarrhea in rural Kenya and evaluated the association of maternal HIV status with *Cryptosporidium* infection among HIV-uninfected children. We enrolled children aged 6 months to 15 years presenting to two Western Kenya district hospitals with acute diarrhea from December 2011-November 2013. Stool samples were tested using standard bacterial culture and microscopy methods for ova and parasites. Demographic and clinical data were collected and child HIV-status ascertained by antibody testing or PCR if <18 months. For this analysis, we included HIV-negative children aged 6 months to 5 years who were accompanied to the hospital by their biological mother. Prevalence ratios (PR) for selected pathogens and 95% confidence intervals (CI) were estimated using relative risk regression. Potential confounders (site, income, household size, treated water, age, height for age z-score [HAZ], breastfeeding, and cotrimoxazole [CTX] use) were included stepwise in a multivariable model. Among 854 HIV-uninfected children with acute diarrhea, median age was 20 months (IQR 10-35), median HAZ was -0.5 (IQR -1.5-0.7), and 10.7% had an HIV-infected mother. Children of HIV-infected mothers were similar in terms of age and household size compared to children of HIV-uninfected mothers, but were more likely to be enrolled at the Homa Bay site (85.6% vs. 51.0%,  $p<0.001$ ), to be stunted (HAZ <-2) (23.0% vs. 14.8%,  $p\leq 0.05$ ), to report recent use of CTX (22.2% vs. 4.0%,  $p<0.001$ ), and household income < 5,000 Kenyan Shillings (KSh) (64.4% vs. 38.0%,  $p<0.001$ ). The prevalence of *Cryptosporidium* was 4.3% (95%CI: 2.9%-5.7%). Children of HIV-infected mothers were >3 times more likely to have *Cryptosporidium* identified than children of HIV-uninfected mothers (11.1% vs. 3.5%, PR: 3.2, 95% CI 1.6-6.4,  $p=0.001$ ). After adjusting for potential confounders, maternal HIV remained significantly associated with *Cryptosporidium* infection in the child (PR: 2.5, 95%CI: 1.2-5.1,  $p=0.014$ ). Prevalence of other diarrheal pathogens did not differ by maternal HIV-infection status. *Cryptosporidium* was more prevalent in children accompanied by HIV-infected mothers. It is possible that HIV-infected mothers are transmitting the parasite to their children and/or that these children are more susceptible to infection.

## O61 Risk factors for giardiasis and parasite assemblages in North West England: a case-control study

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*Giardia duodenalis* is a leading but neglected cause of infectious gastroenteritis worldwide. Very little is known about the epidemiology and true burden of giardiasis in developed countries, largely due to the small number of case-control studies that have been undertaken. We have recently shown that the proportion of infections acquired indigenously, and not through foreign travel, is greater than previously thought. Furthermore, the epidemiology of giardiasis is complicated by the fact that this parasite is a complex of seven genetic assemblages (A-G), or cryptic species, distinguishable only by PCR and DNA sequencing. Humans are predominantly infected by assemblages A and B, and previous studies seem to suggest that these two assemblages may differ in terms of their transmission routes and the illness they cause. However there are inconsistencies in these reported results and more systematically acquired evidence is needed which combines detailed epidemiological data with molecular genotyping. In order to answer these questions, we undertook a case-control study based in North West England to assess 1) the risk factors for giardiasis and 2) whether different parasite assemblages and/or multilocus genotypes were associated with particular clinical/epidemiological features in the patients. We collected socio-demographic, clinical and exposure information from 122 ELISA-confirmed cases of giardiasis and 254 age and sex-matched randomly selected healthy controls. In addition, DNA was extracted from faecal samples from each case and the *Giardia* Assemblage was determined by multilocus sequence typing of four *Giardia* genes (bg, gdh, tpi and ssu). Here we present the results of this study. To our knowledge, this is the first case-control study that has combined a detailed risk factor analysis with *Giardia* genotyping in order to identify possible assemblage-specific transmission routes and differential clinical outcomes.

062

## Six year follow-up of chronic fatigue and irritable bowel syndrome after giardiasis: a controlled prospective cohort study

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**Background:** Some patients experience long term sequels of fatigue and persisting abdominal complaints after *Giardia* infection. The present study aimed to estimate the prevalence and risk of irritable bowel syndrome and chronic fatigue six years after acute giardiasis and evaluate change in prevalence and persistence of these conditions from three to six years after the infection.

**Methods:** This is a controlled prospective cohort study with postal questionnaire follow-up at three and six years after acute giardiasis. In all 748 individuals with laboratory confirmed giardiasis during a waterborne outbreak in Bergen, Norway in 2004 and 878 matched controls responded six years after. 601 exposed and 559 controls responded to both the three and six year questionnaires. The main outcome measures were the prevalence, relative risk (RR), and persistence over time of irritable bowel syndrome and chronic fatigue after *Giardia* infection in a non-endemic setting.

**Results:** The prevalences of chronic fatigue (30.8%) and irritable bowel syndrome (39.4%) in the exposed group six years after giardiasis were significantly elevated compared to controls with adjusted RRs of 2.9 (95% CI 2.3-3.4) and 3.4 (95% CI 2.9-3.9), respectively. Chronic fatigue and irritable bowel syndrome attributable to giardiasis among exposed was 65% and 70.5%, respectively. In the exposed group the prevalence of chronic fatigue decreased by 15.3% from three to six years after *Giardia* infection (RR 0.69; 95% CI 0.62-0.77), while the prevalence of irritable bowel syndrome decreased by 6.7% (RR 0.85; 95% CI 0.77-0.93). *Giardia* exposure was a significant risk factor for persisting chronic fatigue and irritable bowel syndrome from three to six years. Increasing age was a risk factor for persisting chronic fatigue.

**Conclusions:** A high prevalence of irritable bowel syndrome and chronic fatigue was found six years after *Giardia* infection. The prevalences of both conditions decreased over time, indicating that *Giardia* infection may elicit very long term but slowly self-limiting complications.



## O63 Prevalence, genetic diversity and risk factors of *Cryptosporidium* and *Giardia* infections among school children in Lebanon

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*Cryptosporidium* and *Giardia* are protozoan enteroparasites with worldwide distribution that infect the gastrointestinal tract of vertebrates including humans. Both parasites have been recognized as the predominant cause of waterborne and foodborne outbreaks. They are among the major causative agents of gastroenteritis and nutritional disorders in humans, with millions of new cases occurring every year. *Cryptosporidium* oocysts and *Giardia* cysts are infectious immediately upon being excreted in feces and have the potential to be transmitted by fecal-oral route. Due to a common link with poverty especially in developing countries, both pathogens were included in the World Health Organization's Neglected Disease Initiative since 2004. The situation of *Cryptosporidium* and *Giardia* infections is not clear in Lebanon, a developing country often affected by parasitic infections. This study was devoted to determine the prevalence and the genetic diversity of *Cryptosporidium* and *Giardia*, in two children populations with different socio-economic level in Lebanon, as well as the risk factors associated with these two infections. Fecal samples obtained from children were examined microscopically by direct-light microscopy of wet mounts. In addition, modified Ziehl-Neelsen staining as well as molecular tests were done for the detection of *Cryptosporidium* oocysts. Out of 250 children, *Giardia* and *Cryptosporidium* were present in 14.4% and 5.6% respectively according to microscopy examination. Based on molecular tools, the prevalence of cryptosporidiosis cases raised to 10% with predominance of *C. hominis* (74%). Subgenotype analysis of the isolates at the 60-kDa glycoprotein (GP60) locus identified two subtypes IbA10G2 (83%) and IaA18R3 (17%) for *C. hominis* and only one subtype IIaA15G1R1 for *C. parvum* (100%). The study of genetic diversity of *Giardia* is now in progress. Several risk factors such as age, low socio-economic status, eating raw vegetables and fruits, drinking non-treated water, having parents with gastro-intestinal symptoms and presence of abdominal pain were associated with giardiasis. However, cryptosporidiosis was only correlated with having meals outside home and presence of gastro-intestinal symptoms especially diarrhea ( $p < 0.05$ ). This work constitutes the first molecular epidemiology study outlining risk factors associated with cryptosporidiosis and giardiasis in Lebanon.

## CELL BIOLOGY

O64

***Giardia intestinalis*, a highly divergent eukaryote, has a noncanonical cell cycle**

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*Giardia intestinalis* possesses a well regulated cell cycle including timely regulation of cyclin kinase activity, despite lacking an anaphase-promoting complex (APC). We investigated the role of protein phosphatases (PP), PP1 and PP2A in mitosis and found PP1 is necessary for cell cycle progression, whereas PP2A is dispensable. Depletion of PP1 by morpholino-mediated knockdown results in multinucleated cells and fragmented nuclei with aneuploidy, suggesting it regulates both cytokinesis and mitosis. Morpholino-mediated knockdown of INCENP, the activator of aurora kinase (AUK) resulted in mononucleate cells and chromosome mis-segregation; phenotypes complementary to those obtained when PP1 is depleted. We propose that, in the absence of the APC in *Giardia*, PP1 negates the activity of AUK until metaphase, as opposed to the mechanism found in metazoans where AUK is ubiquitinated by the APC and degraded at the end of mitosis. *Giardia* has conserved several MCC components (BUB3, MAD2 and Mps1) but is missing many others. MAD2 is restricted to the cytoplasm during the cell cycle, but Bub3 and Mps1 are associated with the chromosomes. Morpholino knockdown of BUB3, MAD2 or Mps1 results in a lowered mitotic index, chromosome missegregation, and abnormal spindle morphology. During Interphase, MCC knockdown cells have an abnormal number of nuclei, either one nucleus usually on the left hand side of the cell, two nuclei with one of them misplaced from its proper location, or three nuclei. This suggests that the minimalist MCC in *Giardia* plays a major role in regulating many aspects of chromosome segregation including coordination of mitosis between the two nuclei and subsequent nuclear positioning.

O65

## A new technique to study the *Giardia intestinalis* cell cycle

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The lifecycle of *Giardia* alternates between the motile trophozoite and the infectious cyst. The proliferation of *Giardia* trophozoites during an active infection, and the restriction point for the differentiation of trophozoite to cyst are dependent on the tight regulation of the cell cycle. The drugs aphidicolin and nocodazole have been successfully used in the cell cycle synchronization of *Giardia* trophozoites cultures, but these drugs are also associated with an increase in the level of phosphorylated histone H2Ax within the treated cells—which is an indicator of double-stranded DNA breaks. Hence, we applied counterflow centrifugal elutriation (CCE), which is a velocity sedimentation method that separates cells on the basis of their size, shape, density, and DNA content, to fractionate asynchronous *Giardia* cultures. Flow cytometry analysis and estimation of the DNA content in the cells by measuring the relative fluorescence of the nuclei after DAPI staining showed that we were able to obtain fractions of *Giardia* cultures enriched in defined stages of the cell cycle by this method. Furthermore, the cells in these fractions do not have an increase in phosphorylated histone H2Ax compared to control cells. Details of these assays and results from the RT-qPCR on the RNA extracted from these CCE fractions will be presented.

O66

## An Unusual Role for the Giardial Clathrin Heavy Chain?

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In the intestinal protozoan *Giardia lamblia*, intracellular compartment organization appears to be simplified and familiar morphological landmarks and organelles for intracellular trafficking are missing (e.g. the Golgi apparatus or endosomes). Three organelle systems are clearly discernible in trophozoites: the endoplasmic reticulum, mitosomes, and the peripheral vesicles (PVs). PVs are arrayed in a compact layer of resident, ~150 nm compartments embedded mostly in the dorsal cortical area below the plasma membrane. They constitute a highly specialized endosomallysosomal system which acidifies, accumulates fluid phase cargo, and contains hydrolases. Reversible uptake of bulk fluid phase cargo into PVs allows trophozoites to constantly and safely sample their environment to acquire nutrients. Although internalization of membrane-anchored molecules has also been demonstrated, the molecular basis for receptor-mediated endocytic trafficking remains unclear, in particular because no clathrin coated vesicles or pits at the plasma membrane have been identified. Clathrin coats play a key role in higher eukaryotes by forming vesicles at the plasma membrane or the trans-Golgi to capture and deliver cargo for endocytic or exocytic transport. Although in trophozoites clathrin heavy chains (CHC) localize exclusively within the cortical area containing PV organelles, their distribution is not consistent with formation of vesicles. No genes coding for clathrin light chain or many of the classical clathrin co-effectors have been identified in *Giardia*. In addition, using live cell fluorescence imaging we demonstrated that CHC accumulations in the cell cortex have a very slow turn-over. Taken together, this suggests that giardial clathrin has lost its ability to form endocytic transport vesicles in favor of a more static function, possibly in PV morphogenesis. Such a far-reaching reassignment of cellular function entails significant change in the machinery for recruitment and turnover of CHC at membranes. We are currently testing this idea by elucidating the giardial CHC interactome by: (i) generating a comprehensive and validated dataset of CHC-associated proteins, and (ii) exploiting this information to create conditions which interfere with PV function and/or morphogenesis. In addition, we are using EM tomography and super-resolution light microscopy to address the role of static CHC accumulations associated with PVs. The possible exaptation of clathrin function in *Giardia* is an instructive lesson in cellular evolution and provides new insights in to basic principles of membrane coat formation and function.

## O67 Characterization of *Giardia lamblia* Cytochromes b5

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Although it apparently lacks the ability to synthesize heme, *Giardia lamblia* encodes several heme proteins, including a flavohemoglobin [1, 2] and four members of the cytochrome b5 family of electron transfer proteins [3, 4]. Three of the *Giardia* cytochromes b5 (gCYTb5 I-III) are ~15 kDa proteins in which the heme-binding core is flanked by highly charged sequences that differ among each isotype. Recombinant gCYTb5 I-III are expressed in *E. coli* in the heme-bound state, demonstrating that the genes encode functional heme proteins. However, while the properties and roles of cytochromes b5 in other organisms, particularly mammals, are well understood, their roles in *Giardia* are unknown. To address this deficiency in our knowledge we are characterizing the biophysical properties of recombinant gCYTb5s. We previously reported on the properties of gCYTb5-I, including measurement of its reduction potential by cyclic voltammetry, UV-visible spectroscopy, and resonance-Raman spectroscopy [3]. In this work we extend our characterization to gCYTb5-II and III, and use spectroelectrochemistry to measure the reduction potentials of isotypes I, II and III. We confirm the relatively low reduction potential for gCYTb5-I (-175 mV vs the standard hydrogen electrode) by this method and also observe low potentials for gCYT-II and III of -140 mV each. Homology modelling of these proteins suggest that the low reduction potentials of the *Giardia* proteins compared to mammalian isotypes (-100 to +40 mV) is likely due to a more hydrophilic heme environment. As the reduction potentials of electron transfer proteins must be thermodynamically consistent with their redox partners, the gCYTb5s are likely components of electron transfer pathways within *Giardia* that are distinct from those in other organisms.

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## O68 Chromosomes and karyotype of *Giardia intestinalis*: individual cell analysis

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*Giardia* chromosomes belong to the smallest eukaryotic chromosomes (300 nm - 1.5 µm). Using high-resolution field emission scanning electron microscopy we characterized their overall morphology, functional chromatin elements and chromosome segregation during mitosis. Individual cell analysis of *Giardia intestinalis* karyotype revealed aneuploidy and unequal gene distribution in each of the two nuclei both by cytogenetic analysis and FISH detection of selected single-copy genes localized on chromosomes 1-5. The probe binding pattern indicates variable chromosomal ploidy among individual nuclei of a trophozoite cell and among trophozoites, resembling a mosaic-type of aneuploidy, described recently in *Leishmania* sp., or/and ongoing heterologous recombination and chromosome rearrangements. Karyotype heterogeneity in laboratory lines and clones derived from the WB isolate (ATCC 30957), i.e. the presence of different chromosome numbers, minor karyotype variants, and a karyotype change during long time cultivation of certain WB lines, indicate high levels of chromosome instability likely due to chromosome missegregations. This process can be tolerated in *Giardia* by lack of the functional spindle assembly checkpoint and absence of key molecules regulating ploidy control. In contrast, a stable aneuploidy with a minimal karyotype dynamics has been observed in other *Giardia* lines. Aneuploidy in *Giardia* may increase its genetic variability and play a prominent role in cellular adaptations to host and environmental conditions.

O69

## Cytosolic Iron/Sulphur cluster Assembly machinery in *Giardia intestinalis*

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Eukaryotes combined several pathways for Iron/Sulphur (FeS) cluster synthesis to effectively deliver clusters to apo-proteins in different cellular compartments. The Cytosolic Iron/Sulphur cluster Assembly (CIA) pathway is ubiquitous in all eukaryotic cells and seven proteins in this pathway have been identified thus far (Tah18, Dre2, Nbp35, Cfd1, Nar1, Cia1 and MMS19).

In yeasts, it has been shown that FeS clusters formation via CIA pathway is fully dependent on mitochondrial FeS cluster assembly machinery (ISC). Atm1 transporter in inner membrane and Erv1 protein in intermembrane space of mitochondria were found to be involved in this connection. Nevertheless, the mechanism of export of yet unknown precursor of FeS cluster across the outer membrane of mitochondria remains unknown. Tah18 and Dre2 proteins were both localized in cytoplasm and mitochondria of yeast; however their possible involvement in CIA and ISC linking has not been clarified so far.

As an adaptation to anaerobic environment, *Giardia intestinalis*, a member of phyla Metamonada, reduces its mitochondria to mitosomes. The only pathway conserved within this organelle is FeS clusters synthesis mediated by ISC. Thus, the key function of this organelle is most likely the cooperation with CIA pathway to mature FeS proteins in the cytosol. Surprisingly Atm1, Erv1 and Dre2 proteins are absent in *Giardia* genomic data, and only Tah18-like protein, Nbp35, Nar1 and Cia1 were identified.

Here we investigated (i) the origin of Tah18-like proteins in Metamonada (ii) the presence of Dre2 in Metamonada and (iii) how ICS and CIA pathways are linked in *Giardia*. Phylogenetic analysis revealed that two *Giardia* Tah18-like proteins evolved from Pyruvate: NADP oxidoreductase, and do not cluster with Tah18 orthologues. The analysis also suggested that N-terminal part of PNO was lost, and replaced by hydrogenase modul in ancestor of Metamonada and secondarily lost in *Giardia*. Moreover we were unable to identify any orthologue of Tah18 and Dre2 protein in other Metamonada. Next we tested the topology of three Nbp35 giardial paralogues. One paralogue revealed cytoplasmic distribution, while other two have unique dual mitosomal/cytoplasmic localization similar to Dre2 protein in other eukaryotes. We speculate that dual localization of Nbp35 proteins in *Giardia* and Dre2 proteins in other eukaryotes might be important for ISC and CIA connection.

## 070 Molecular analysis of the mitosomal protein import in *Giardia intestinalis*

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The endosymbiotic acquisition of mitochondria was a key event in the early eukaryotic evolution. During the transformation of the endosymbiont, highly effective system for the targeting and the import of mitochondrial proteins from cytosol has evolved. In yeast and mammalian cells, the protein import machinery is very complex, comprising more than 30 protein components. In unicellular anaerobic parasite *Giardia intestinalis*, mitochondria are highly reduced to tiny organelles called mitosomes, which have completely lost their energy metabolism as well as the genome. All proteins thus have to be imported from the cytosol. Surprisingly, mitosomal protein import machinery comprises only four components known so far. This extreme simplification makes *Giardia* an exciting model for studying the mitochondrial/mitosomal protein translocation. Given that the protein sequences of many *Giardia* proteins are highly divergent, bioinformatic approaches usually fail to identify clear homology to known mitochondrial components. Hence, it is crucial to develop more efficient tools to identify the proteins directly. Here, we established a new method for isolation of interacting proteins in *Giardia*. We created a system for highly specific in vivo protein biotinylation using bacterial enzyme biotin-ligase. Biotinylated target protein was crosslinked and purified by streptavidin affinity capture. We made use of the only known inner membrane component of the protein import pathway Pam18 as a biotinylation target. By purification of its interacting partners we managed to identify a highly divergent homologue of Tim44 and several other new mitosomal proteins. Moreover, we used this new biotinylation system to study the dynamics of mitosomal transport and cellular trafficking in vivo. Our results revealed that mitosomal matrix chaperone GrpE undergoes posttranslational (mitochondria-like) targeting. To address the question of whether the proteins enter the import pathway in unfolded state (such as in mitochondria), we established a system using the mouse DHFR domain, folding of which can be induced by the addition of antifolates. In *Giardia*, the mitosomal localization was fully abolished upon the DHFR folding, showing that proteins must retain the unfolded state for the mitosomal delivery.



## 071 Specific targeting of axonemal protein GAX to flagella of *Giardia intestinalis*

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The axoneme of eukaryotic flagella consists of hundreds of proteins that are assembled in a complex flagellar architecture. The core structure consists of 9 microtubular doublets with dynein arms and a central microtubular pair. TAX-2 protein has been recently shown to be associated with axoneme of *Trypanosoma brucei* and its ablation caused axonemal defects and impaired cytokinesis in bloodstream trypanosomes. Although the exact role of TAX-2 is unknown, the TAX-2 orthologues are widely conserved in flagellated eukaryotes including *Giardia intestinalis*.

TAX-2 orthologue in *G. intestinalis* named GAX consists of N-terminal domain with homology to recently described soluble cytochrome b5 (71 AA), central ubiquitin-like domain (69 AA) and conserved C-terminal part without known homology. Interestingly, although cytb5 domain of GAX displayed over 30% identity with other giardial cyt b5 proteins, it lacks heme binding side. Indeed, we confirmed that recombinant GAX is unable to bind heme. To validate expected flagellar localization, GAX was expressed with HA tag in *G. intestinalis*. Immunofluorescence microscopy revealed association of GAX with naked axonemes as well as in axonemes of complete flagella, however they were absent in basal bodies. We also did not observe presence of GAX in other microtubular structures such as adhesive disk and medial bodies. When we induced *Giardia* encystation, GAX remains associated with axonemes during encystation and in cysts, although, some signal was observed also outside of axonemes. As this axonemal protein is highly conserved in eukaryotes, we overexpressed *Trichomonas vaginalis*, *T. brucei* and *Homo sapiens* orthologues in *G. intestinalis* to test specificity of its targeting to the axoneme. Surprisingly, none of heterologous proteins was efficiently targeted to giardial axonemes. Similarly, overexpression of the same set of GAX orthologues in *T. vaginalis* led to efficient targeting of this protein to axonemes only when *T. vaginalis* gene was used, while all heterologous proteins were observed predominantly in the cytosol. Finally, we constructed mutated GAX with deleted C-terminal domain of 19 AA. This deletion completely prevented association of GAX with *G. intestinalis* axonemes suggesting that this part participates in correct GAX targeting. Further studies will be focused on detail mapping of GAX targeting signals, and identification of GAX flagellar partners.

072

## Unravelling the molecular basis for the elusive mitosomal protein import function in *Giardia lamblia*

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*Giardia lamblia* lacks bona fide mitochondria and instead harbors highly reduced and diverged mitochondrial-related organelles (MROs) termed mitosomes. These organelles contain no DNA, but import nuclear encoded proteins both in a transit peptide-dependent or -independent manner, presumably via functionally conserved mitochondrial protein import pathways. Despite direct evidence for a canonical protein import function, a poorly conserved TOM40 homolog is the only subunit of the mitochondrial protein import complexes (TOM/TIM) identified to date, primarily due to the high sequence degeneracy. The main goal of this work is to generate a comprehensive list of TOM40-interacting, and other non-conserved MRO proteins. We hypothesize that this information will not only help unraveling the molecular basis for protein import into giardial mitosomes, but also lead to (re)discovery of components that were derived beyond recognition during reductive evolution of *Giardia*. To test this and to identify the complete import machinery in giardial MROs, we used epitope-tagged TOM40 as bait protein and performed co-immunoprecipitation experiments using various degrees of reversible cross-linking followed by protein tandem mass spectrometry. An initial set of 78 identified proteins was narrowed down to 41 high-quality specific hits, using reverse coimmunoprecipitation and subtractive in silico filtrations. The dataset includes proteins involved in membrane translocation, Fe-S synthesis, chaperonins, and a significant number of novel proteins whose localizations and functions are now under investigation. Specifically, organelle-specific localization of 8 hypothetical proteins was confirmed, 6 of which are novel and were not identified in previously published proteomes. This is indicative of various additional functions yet to be discovered for these unique relic organelles. With this data we are building a comprehensive interactome of the inner and outer mitosome membranes. In addition, we are developing novel organelle membrane markers for analysis of interactions between MROs and other cellular components, as well as for analysis of organelle dynamics.

## GENOMICS, TRANSCRIPTOMICS & PROTEOMICS

### 073 *Cryptosporidium* AP2 proteins and evolution of gene networks in the Apicomplexa

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We present a comprehensive analysis of putative transcription factors in *Cryptosporidium parvum*. Apicomplexans appear to have acquired the AP2 (ApiAP2) family of transcriptional regulators and have a notable absence of canonical enhancers. We used Hidden Markov Models (HMMs) and phylogenetics to show that apicomplexan and perkinid AP2 domains cluster distinctly from other chromalveolate AP2s. We determined the DNA-binding specificity of *Cryptosporidium* AP2 domains. We combined the experimental binding results with motif conservation upstream of coregulated *C. parvum* gene clusters to construct putative AP2 regulons. We show that *C. parvum* ApiAP2 genes are expressed across the in vitro lifecycle; however, orthologous ApiAP2 expression has been rearranged relative to the malaria parasite *P. falciparum*. Few putative downstream gene targets are conserved between these species, suggesting extensive ApiAP2 network rewiring during evolution. *C. parvum* AP2 domains display reduced binding diversity with respect to *P. falciparum*, with multiple domains capable of binding the 5'-TGCAT-3', 5'-CACACA-3', and G-box motifs (5'-G[T/C]GGGG-3'). Many overrepresented motifs in *C. parvum* upstream regions do not appear to be AP2 binding motifs. We hypothesize that *C. parvum* is less reliant on ApiAP2 regulators than other studied apicomplexans in part because it utilizes E2F/DP1 transcription factors. *C. parvum* may provide clues to the ancestral state of apicomplexan transcriptional regulation.

## 074 Epigenetics and Signal Transduction in *Cryptosporidium parvum*

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The complex life cycle of *Cryptosporidium parvum* is regulated by a unique signal transduction system as well as distinctive epigenetic mechanisms. To study both systems, we carried out a large scale study based on the following steps: First, we profiled the kinome and epigenome of this apicomplexan parasite, and compared them against other parasites. Next, we expressed, purified and characterized a number of *C. parvum* protein kinases (PK), as well as bromodomains (BRD) and histone methyltransferases (HMT) as recombinant proteins. Thirdly, we screened the proteins against known inhibitors (for each protein family) and, in the case of the BRDs and HMTs, a library of histone peptides. Finally, we crystallized a number of the kinases and bromodomains, and obtained their structures. The results of our study include: phylogenetic catalogues of the *C. parvum* kinome and epigenome; inhibitors of specific *C. parvum* PKs (including those active against the parasite), BRDs and HMTs, crystal structures of a number of PKs, BRDs and HMTs. These results enable us to present hypotheses about the signal transduction system of *C. parvum* and its mechanism of epigenetic regulation. Furthermore, we present a number of potential chemical probes which may be used to characterize the function of the proteins in our study. These probe molecules will be made available to interested researchers.

## 075 Label-free quantitative proteomics in *Giardia duodenalis*

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*Giardia duodenalis* is a protozoan parasite and major contributor worldwide of diarrhoea and gastroenteritis in vertebrates, including humans. With advances in genetic epidemiology, quantitative proteomics presents a unique opportunity to analyse and quantify protein expression across *Giardia* life stages, genetic assemblages and in host-parasite interactions. We have compared both in-gel and in-solution preparation of protein extracts and optimised a method utilising filter aided sample preparation (FASP) for digestion and gas-phase fractionation (GPF) for peptide separation. Proteins were identified using LC-MS/MS spectra that were acquired on a LTQ-XL linear ion trap mass spectrometer and peptides were matched individually against the GiardiaDB.org 2.5 [1] release of *G. duodenalis* WB genome for Assemblage A [2]. Protein abundance was quantified using spectral counting, which makes our studies the first to utilise label-free quantitative proteomics in this parasite. To date we have analysed proteomes of eight Assemblage A human or animal strains of *G. duodenalis* to identify proteins of differential abundance for intra-Assemblage variation and host origin. We are currently in the process of applying our label-free methods to investigate the proteome during *Giardia* encystation and excystation, as well as secreted proteins. In this capacity, we believe that quantitative proteomics is a valuable tool for confirming and exposing the subtleties within strain diversity, as well as complementing advances in gene identification, transcript expression and genome data towards a systems biology approach in understanding *Giardia*.

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**076**      **Oxygen metabolism and nitroheterocyclic drug resistance in *Giardia* – common themes and novel bioinformatics insights**

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Giardiasis is almost exclusively treated with the nitroheterocyclic drug metronidazole (Mz), and to a lesser extent with the benzamidazole (Bz) drugs albendazole or mebendazole. Given these limited treatment options, understanding the mechanisms of drug resistance is of paramount importance. Although cases of Bz treatment-resistant infections are rare, treatment failure of Mz is as high as 20% in some populations, and Mz resistance has been confirmed in clinical isolates and laboratory cultures. Despite the occurrence of failed Mz-treatments, the molecular mechanisms underpinning resistance are poorly understood. Previous research into drug-resistance has centered heavily on pyruvate:ferredoxin oxidoreductase (PFOR) and ferredoxin I, which were the first reported Mz-activating enzymes in *G. duodenalis*. Although some Mz-resistant isolates show decreased activity or transcription of these enzymes, other components of redox and metabolic pathways may also be involved and have not been extensively explored. Characterizing such pathways in *G. duodenalis* is made challenging by the large number of 'hypothetical' proteins currently predicted to be encoded in the genome. Using advanced bioinformatic tools, we mined the hypothetical proteins in the WB strain and proposed potential molecular functions for an additional ~300 sequences, including a number with inferred roles in redox and/or epigenetic regulation. We present here a summary of these molecules, as well as known *Giardia* metabolic pathways in the context of current knowledge on the mechanisms of Mz-resistance in this species, providing a holistic view of drug-resistance in *Giardia* and presenting a foundation for extensive system-wide exploration of these mechanisms.

## 077 The Cre/loxP system in *Giardia lamblia*: Genetic manipulations in a tetraploid protozoan

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The study of molecular and cellular processes in *Giardia lamblia* suffers from the severe limitations of applying reverse genetic approaches, most notably knock-out strategies, to a tetraploid genome.

We attempted to address this important obstacle in *Giardia* molecular research techniques by developing a *Giardia*-tailored Cre/loxP system. The Cre/loxP method is a fascinating and valuable bacteriophage P1-derived tool that has revolutionized genetic and cell biological research in many organisms.

In this report, we demonstrate that the Cre-recombinase can be used to genetically fix insertions/deletions in *G. lamblia* chromosomes and to recycle selectable markers, allowing for multiple rounds of transfection of the same cell line. We made use of this new-found flexibility to develop dual reporter *Giardia* lines, where the simultaneous labeling of two subcellular compartments was achieved using a single positive selection marker. This in part obviates the need for the often laborious production of homologous antibodies and avoids use of heterologous antibodies which have been shown to give rise to labeling artefacts.

Our data provides proof-of-concept for the successful application of the Cre/loxP method to *Giardia* cells. Furthermore, our findings in a model tetraploid protozoan such as *Giardia lamblia* offer exciting prospects for the functional gene and protein investigation via more complex and versatile genetic manipulations of this and of other polyploid protozoans.

## 078 The genome and in vivo transcriptome of *Giardia muris*

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Natural infective models of *Giardia* infection has been used to study the immunological response to infection in the host for many years. *Giardia muris* infects mice and produces a disease similar to *G. intestinalis* infection in humans. Although *G. muris* has been used extensively to study immunological aspects of giardiasis, very little is known about the genome and biology of this parasite since it is recalcitrant to axenization. To promote and enable research on *G. muris* we have generated a draft genome sequence of *G. muris* Roberts-Thompson. This genome represents the first genome of *Giardia* outside of the *G. intestinalis* assemblages. We have noticed large differences in genome synteny and in large gene families compared to *G. intestinalis*. We have also generated the first genome-wide expression datasets from *Giardia* trophozoites isolated directly from the gut of a host animal using immunoaffinity techniques. Strand-specific RNAseq and small RNA data generated from in vivo *G. muris* trophozoites allowed an unprecedented view of the regulatory and transcriptional pattern during infection in a natural host. High level transcription of genes associated with the encystation pathway was detected, indicating that a subset of the isolated population had entered into differentiation pathway. The draft genome of *G. muris* will enable comparative studies of *Giardia* biology and create a resource for in vivo infection studies.



## 079 Investigation of Waterborne Outbreaks of Giardiasis By Whole Genome Sequencing

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While waterborne outbreaks of giardiasis have decreased significantly, the western Canadian province of British Columbia (BC) still has high reported infection rates. Previous studies of *Giardia* isolates have been carried out using available tools such as iso-enzyme analysis. The information retrieved using these tools however has been limited by several factors including their depth of discrimination and the availability of parasite strains. In this study, trophozoite DNA was extracted from cultured strains retrieved previously using in vitro and in vivo methods of excystation. Next Generation Sequencing (NGS) technology was used to characterize genomic variations among select *Giardia* strains from human, animal and water sources. Genomes of over 50 *G. intestinalis/lamblia* strains, including outbreak and non-outbreak related strains, were sequenced using Miseq (Illumina) and data assessed for quality then analysed using established in-house bio-informatic pipelines. We report on the possible use of these microbial genetic tools to better understand the transmission of spread of giardiasis in BC as well as the biology of this important parasite.

O80

## The Genome of *Spironucleus salmonicida* Highlights a Fish Pathogen Adapted to Fluctuating Environments

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*Spironucleus salmonicida* causes systemic infections in salmonid fish. It belongs to the group diplomonads, binucleated heterotrophic flagellates adapted to micro-aerobic environments. Recently we identified energy-producing hydrogenosomes in *S. salmonicida*. Here we present a genome analysis of the fish parasite with a focus on the comparison to the more studied diplomonad *Giardia intestinalis*.

We annotated 8,067 protein coding genes in the ~12.9 Mbp *S. salmonicida* genome. Unlike *G. intestinalis*, promoter-like motifs were found upstream of genes which are correlated with gene expression, suggesting a more elaborate transcriptional regulation. *S. salmonicida* can utilise more carbohydrates as energy sources, has an extended amino acid and sulfur metabolism, and more enzymes involved in scavenging of reactive oxygen species compared to *G. intestinalis*. Both genomes have large families of cysteine-rich membrane proteins. A cluster analysis indicated large divergence of these families in the two diplomonads. Nevertheless, one of *S. salmonicida* cysteine-rich proteins was localised to the plasma membrane similar to *G. intestinalis* variant-surface proteins. We identified *S. salmonicida* homologs to cyst proteins and showed that one of these is functional when expressed in *Giardia*. This suggests that the fish parasite is transmitted as a cyst between hosts.

The extended metabolic repertoire and more extensive gene regulation compared to *G. intestinalis* suggest that the fish parasite is more adapted to cope with environmental fluctuations. Our genome analyses indicate that *S. salmonicida* is a well-adapted pathogen that can colonize different sites in the host.

**O81      The genome is not enough: Expanding our knowledge base through the genome sequencing of multiple clinical isolates of *Cryptosporidium* and *Giardia* from Scandinavia**

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In Sweden, as well as in many other countries, *Cryptosporidium* and *Giardia* infections are notifiable. Several ongoing projects aim to improve our understanding of the prevalence, diversity of species/strains, and sources of contamination. Fuelled by a number of high profile outbreaks in Sweden during the last decade, a coordinated governmental agency initiative started a large genome sequencing project to gain detailed knowledge on the two parasites in a combined human and animal welfare perspective. We aim to sample zoonotic as well as host specific species with the ultimate goal to improve typing of the parasites in the future. Other goals cover the biology of the parasites, mainly focusing on pathogenicity, population genetics and evolution.

During the first phase of the project, the diversity of *Cryptosporidium* and *Giardia* in Sweden is surveyed by collection of clinical faeces samples from a broad range of human and animal hosts. Strains are selected for whole genome sequencing to cover as many strains across both parasites as possible, although some isolates of similar genotype will also be chosen to determine the variation within species/assemblages/genotypes as well. We are then implementing next generation sequencing (NGS) to sequence each genome. We are building a bioinformatic platform to optimize data analysis. All sequences will ultimately be deposited in publically available databases, and we have initiated a dialog with EuPathDB in order to effectively present our data to the *Cryptosporidium* and *Giardia* communities. The goal is to sequence the genomes of 60 clinical isolates of *Giardia* and *Cryptosporidium* sampled in the Nordic countries. The genomic data will be further explored to answer a broad array of questions in population dynamics, host specificity and pathogenicity and organism biology but also be used to design sets of genetic markers to improve current methods for detection and typing.

082

**The numbers game: Development of a method to obtain high quality material from ALL *Cryptosporidium* and *Giardia* samples for genome sequencing**

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Working with *Cryptosporidium* and *Giardia* provides several challenges in obtaining sufficient material from both a basic research and diagnostics perspective. The lack of extensive genome data is a major bottleneck in our ability to further our understanding of both parasites. Difficulties in establishing cultures, low parasite load from host samples, and inhibitory contaminating material, are but a few of the factors which limit our ability to develop new assays, and to better understand the parasites basic biology. The aim of our project is to develop robust and flexible methods allowing us to sequence the genomes of *Cryptosporidium* and *Giardia* from clinical isolates, irrespective of sample matrix and quantity. In order to achieve this, we have been working to establish and optimize a protocol which would allow us to concentrate and retain as many parasites as possible, whilst removing background material (particles and inhibitory factors) as well as contaminating DNA (mammalian host, bacterial, fungal, etc). The next stage utilizes whole genome amplification systems to obtain large quantities of DNA. As a result, we have a system in which we are able to purify, extract, and amplify sufficient *Cryptosporidium* or *Giardia* DNA to facilitate whole genome sequencing and annotation of clinically derived samples, enabling us to establish a database of isolate information which can be mined for further investigation.

## O83 Transcriptional analysis of *Giardia intestinalis* encystation using RNA seq

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Differentiation into infectious cysts through the process encystation is crucial for transmission and survival of *Giardia intestinalis* in the environment. Hitherto the majority of efforts to explain the encystation process have focused on the early events of the process like formation of encystation-specific vesicles (ESVs), leaving many aspects of late encystation poorly defined. In order to study the late stage of encystation we tested different published encystation protocols and developed a protocol using a modified high-bile encystation medium. This protocol generated a much higher yield of mature, 16N cysts after 24 hours, compared to the standard two-step method. Transcriptional changes during differentiation from trophozoites to cysts in the new medium were studied using RNA sequencing (RNA seq). The two early time points (1.5 and 7 h p.i) were compared to existing transcriptional data generated by microarray and SAGE technology thereby generating a consensus table of up-regulated genes using different encystation protocols and techniques. The consensus table showed that only 13 of the around 6,000 *G. intestinalis* protein encoding genes are consistently up regulated early during encystation. Five genes with unknown function found in the consensus table were further specifically characterized by localization and expression studies. The largest transcriptional changes were seen in the late part of encystation (22 h p.i) and the majority of the highest up-regulated genes at this time point encode hypothetical proteins. Several of these were epitope tagged and localized to gain information of new important proteins involved in the differentiation process. We found three proteins that localizes to the nuclei during the late stage of encystation, four proteins localizes to the cyst wall membrane and another three proteins seem to localize to a cytoskeleton-associated structure in cysts. The localization of these proteins will be studied during excystation since they are likely to play an important role also in this process. We also detected a switch of variant surface proteins (VSPs) in the late phase of encystation. This occurred at the same time as nuclear division and DNA replication, suggesting a potential link between the processes. Our data gives a starting point from which it is possible to further explore important processes occurring in the later part of encystation to gain information about this vital process for parasite survival.

O84

## Transcriptional profiling of *Giardia intestinalis* Response to Oxidative Stress

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*Giardia intestinalis* is microaerophilic parasite that causes diarrhea in humans. Inside the intestines, the parasite is exposed to reactive oxygen species (ROS) produced by the host intestinal epithelial cells in addition to oxygen concentrations reaching up to 46  $\mu$ M. Although *Giardia* lacks mitochondria and several traditional, eukaryotic ROS detoxification enzymes, it can survive in the host's intestine and complete its life cycle. In order to understand how *Giardia* copes with oxidative stress the reference isolates, WB and GS/M, were tested against different concentrations of hydrogen peroxide (3mM to 100mM for 1h) and their transcriptomes were analysed by paired end RNA Seq (at 150 mM hydrogen peroxide, = 99.5% trophozoite viability). Phenotypic differences were seen between the two isolates; GS/M tolerated hydrogen peroxide concentrations of 3 mM while concentrations above 1 mM killed the WB isolate. Transcriptional changes were also compared during growth under both aerobic and anaerobic conditions in *Giardia* growth medium, TYDK, and during interaction with differentiated Caco-2 cells in DMEM. The results indicate that *Giardia* response to oxidative stress might play an important role in isolates' virulence and disease outcome.

## O85 Whole Genome Sequence Analysis of a Clinical Isolate of Zoonotic *C. parvum*: isolate UKP1

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*Cryptosporidium parvum* is one of the two major species of *Cryptosporidium* that infect humans. *C. parvum* is considered zoonotic and has a wide host range with cattle being the predominant reservoir host. However, some subgenotypes of *C. parvum*, such as the IIc gp60 subgroup have not been found in animal hosts and are considered to be anthroponotic. Whole genome sequencing (WGS) provides a means for comparing isolates to identify markers important in distinguishing routes of transmission and potential virulence traits for better epidemiological analysis and risk assessment. The objective of this study was to compare the WGS of a clinical isolate of *C. parvum* with the reference IOWA isolate, and to identify regions of the genome that can be used as diagnostic markers. UKP1 was isolated at the UK Cryptosporidium Reference Unit and identified as gp60 type IIaA17G1R1 (GenBank # JX971701). This subtype has a global distribution and has been observed in cattle, humans and pigs. Oocysts were semi-purified using salt flotation and hypochlorite treatment, and DNA was whole genome amplified prior to 454 sequencing. Paired-end reads were reference mapped to the IOWA II genome and comparison with the UKP1 genome revealed a high degree of homology in micro- and mini-satellite markers used previously for population and epidemiological analysis. Identical sequences were observed between UKP1 and IOWA for markers ML1, ML2, MS5, MS9, TP14, MSA, MSB, MSC, MSD, MSE, MSF, MSG, MSI, MSK, 5B12, MM5, MM18. No differences were observed for 2 of 4 polymorphic markers that we previously identified using cattle isolates of *C. parvum*, markers MS01 and MS03. The sequences of gene markers cgd1\_650, cgd3\_3370, cgd6\_5260 were recently shown to be more similar to *C. hominis* in the clinical *C. parvum* isolate (TU114) of the IIc subgroup that circulates in humans, compared with the zoonotic *C. parvum* isolates. In our study, the UKP1 sequences of these 3 markers were identical to the IOWA isolate. Some divergence between UKP1 and IOWA was observed including insertions in repeat regions of CP492, 1887, MS02 and MS04, and other sites of the genome. We are currently generating Illumina sequence data of UKP1 to be consolidated with the 454 sequence for error-correction of homopolymer tracts, and to provide greater depth of coverage. Genomic sequence data will aid in understanding the population structure of *Cryptosporidium* and provide new tools for improved source attribution and risk assessments.

## 086 Whole genome sequencing of *Cryptosporidium* spp. prepared directly from human clinical samples.

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Next generation sequencing (NGS) has the potential for increasing our knowledge of the biology, transmission, virulence and epidemiology of *Cryptosporidium* spp. and so improve diagnosis, control and treatment of cryptosporidiosis, especially if isolates can be routinely sequenced. However, significant challenges exist for generation of whole genome sequence data from stools: i) NGS methods require significant amounts of genomic DNA and stool samples received by diagnostic laboratories are limited in volume; ii) stools contain relatively small numbers of *Cryptosporidium* oocysts amongst very large amounts of non-target organisms, mainly bacteria, which is problematic for production of the purity of genomic DNA required for analysis; iii) there is a lack of a suitable culture system capable of producing sufficient yields of parasites. Genome studies published to date have utilised oocysts propagated from either experimentally infected animals or repeatedly harvested from natural animal infections.

To provide an ethical and sustainable alternative for widespread investigation of isolates causing human cryptosporidiosis we have developed a genomic DNA preparation protocol for stools. The method has the following characteristics which specifically address the above problems: enhanced purification of oocysts using saturated salt-flotation followed by immunomagnetic separation, degradation of non-*Cryptosporidium* DNA with sodium hypochlorite treatment prior to DNA extraction, and use of the Nextera XT library preparation kit (Illumina) which is capable of processing as little as 1 ng of DNA. In the first instance we successfully sequenced the genomes of a *C. hominis* (IbA10G2), *C. parvum* (isolate from stools). Sequencing was achieved covering 100% of the *C. parvum* reference genome at 51.8x depth and 98% of the *C. hominis* reference genome at 34.7x depth. When compared with the reference genomes, 90.8% of the *C. parvum* and 89.4% of the *C. hominis* reads aligned. Sequencing *C. viatorum* covered 89% of the *C. hominis* reference genome at 51.9x depth and 66.8% reads aligned. The method has been applied to generate further sequences for other subtypes and species from stools.



## POPULATION GENETICS

### O87 Cryptic genetic exchange in human Giardiasis

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The *Giardia* population genetic structure is thought to be clonal, and isolates are grouped into 8 recognized genotypic clusters or 'assemblages', based on limited molecular genetic analyses using highly conserved loci. Assemblages A and B are zoonotic, whereas C, D, E, F, G, and H are defined as host restricted. In the absence of a molecular toolset designed to assess extant genetic diversity or whether natural isolates of *Giardia* undergo genetic exchange, we identified 50 single-copy genes among the publically available genomes of *Giardia* assemblages A, B and E that range in their polymorphic diversity. Because *Giardia* possesses a highly reduced eukaryotic genome (12-14Mb in size) with remarkably few introns, we used the following criteria to develop a suite of genetic markers that represent a robust and comprehensive MLST scheme for characterizing *Giardia* population genetic diversity: single-copy genes not located in subtelomeric regions of the parasite's five chromosomes that were either under purifying, neutral or positive selection. Ten genetic loci were ultimately selected, two for each of the 5 chromosomes, to assess intra- and inter-specific population genetic diversity among 32 A and B Assemblage isolates. Isolates from Peru, Egypt and Afghanistan were compared against isolates collected across North America, which comprised the majority of samples. Using published genetic markers (gdh, tpi, bg, 18S), all 32 isolates segregated into one of the 4 distinct A or B Assemblage sub-types within the *Giardia* taxon. When analyzed using the 10 MLST genetic markers, 24 distinct multi-locus genotypes were resolved. Phylogenetic trees and genealogy networks were constructed to infer the true genetic relationships among the isolates analyzed. According to the tree topologies, a high level of allelic diversity was identified that resolved into five distinct "allelic types" using a robust 85% bootstrap cut-off. At any given locus, a maximum of three allelic types was identified in Assemblage A versus two allelic types in Assemblage B. The inheritance pattern of the allelic types across the genetic loci identified highly reticulated topologies, examples of both inter- and intra-specific recombination, and supported a high level of genetic exchange. Our data is consistent with the majority of isolates existing as intra-Assemblage admixtures of the three (A) or two (B) allelic types. Importantly, we identified 4 isolates (12%) that were inter-Assemblage recombinants based on the inheritance of alleles across the 10 loci. Our data support a model whereby genetic exchange is frequent among natural *Giardia* isolates.

**O88**      **Population structure of natural and propagated isolates of *Cryptosporidium parvum*, *Cryptosporidium hominis* and *Cryptosporidium meleagridis*.**

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The three protozoan species *Cryptosporidium parvum*, *C. meleagridis* and *C. hominis* (phylum Apicomplexa) are enteric pathogens of humans. The former two species are zoonotic and the latter is thought to infect only humans. The limited resolution of conventional PCR-based genotyping methods has likely produced an incomplete view of how these parasites are transmitted as minority subpopulations often remain undetected. To better characterize the structure and transmission of natural and laboratory-propagated isolates, we analyzed a diverse collection of archived human and animal isolates of *C. parvum*, *C. hominis* and *C. meleagridis* by deep-sequencing PCR products amplified from a polymorphic coding sequence on chromosome 1. Thousands of 200-nucleotide sequences obtained from each isolate were initially screened to reduce the frequency of sequencing errors. Screened sequences were aligned and analyzed to compare the diversity among species and samples, to assess the impact of laboratory propagation on population complexity and to identify taxonomically mixed isolates. Contrary to our expectation, repeated propagation in animals did not reduce alpha diversity nor was diversity associated with host species. Significantly, most samples included sequences from different species. The presence of *C. hominis* alleles in *C. parvum* and *C. meleagridis* isolates confirms sporadic reports of mixed isolates and raises the possibility that the host range of *C. hominis* is broader than typically assumed. Using PCR chimera detection software, we found a very unequal distribution of putatively recombinant sequences among isolates. In an anthroponotic *C. parvum* isolate (GP60 IIc genotype) 90% of the sequences were identified as likely *C. parvum* x *C. hominis* recombinants. This observation raises interesting questions about the emergence of genetically distinct *C. parvum* subpopulations.

# Poster Presentations

P1

## **Application of two different concentration and detection methods for the detection of *Giardia* and *Cryptosporidium* in surface water samples in Iran**

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Effective techniques for detecting contaminated (oo)cysts in water are important to prevent and control the contamination. Sucrose flotation (SF) technique is generally used for separating organisms by using their different specific gravity. It is effective and cheap but time consuming as well as requiring highly skilled personnel. Immunomagnetic separation (IMS) method has been widely employed recently due to its efficiency, but, it is costly. A widely used method for detection of (oo) cysts in environmental samples is immunofluorescence assay (IFA), which is time consuming and subject to variations in sensitivity. Therefore new molecular tools must be applied.

Totally 26 surface water samples have been collected from 13 sampling points from rivers in north of IRAN. From each sampling point, two samples in same time collected. 13 samples concentrated by the IMS and 13 samples by the SF method. For each concentrated sample, IFA and PCR assays applied used for the identification of *Cryptosporidium* and *Giardia* (oo)cysts. 10 out of 13 samples were *Cryptosporidium* positive and 9 out of 13 samples were *Giardia* positive by IMS/PCR. By using IMS/IFA, similar SF/IFA, 3 out of 13 and 5 out of 13 samples were positive for *Cryptosporidium* and *Giardia* respectively. Two out of 13 and 3 out of 13 samples were positive for *Cryptosporidium* and *Giardia* respectively by SF/PCR.

Results have shown that IMS/PCR appears to be more sensitive than other methods used in present study for detecting *Cryptosporidium* and *Giardia* in water concentrates. There was no difference in detection of *Cryptosporidium* and *Giardia* by SF/IFA and IMS/IFA. So concentrate by SF and IMS methods give same results when IFA method used for detection. IMS/PCR gives better results than SF/PCR for both parasites that could be due IMS was more successful to remove PCR inhibitor than SF method.

# Poster Presentations

## P2 **Historical facts on the methodology development for pathogenic protozoa in water: conclusions and considerations**

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The parasitic protozoa *Cryptosporidium* and *Giardia* constitute the leading causes of waterborne enteric disease outbreaks worldwide. Detection methods from water have changed considerably over the last 35 years. Sample concentration cartridge filters and flat membrane filtration of the mid '80s were substituted in the mid 90's by new membrane types and different methods (flocculation, continuous flow centrifugation). Hollow fiber ultrafiltration, cartridge and compressed foam filtration in early 2000' provided researchers with alternative concentration techniques. Separation has been achieved by density gradient techniques since mid 80's. The introduction in the late 90's of immunomagnetic separation and its approval by the USEPA method 1623 in 2003, established it as the preferred method for final purification of the (oo)cysts. Detection was originally almost exclusively performed by microscopic methods. In the 1990s techniques such as flow and laser scanning cytometry emerged and are still often used. The advent of PCR and FISH a few years later allowed not only the detection, but also the differentiation between human and animal species. More recently LAMP has been introduced. Recovery efficiency has been reported as early as 1983. Following the USEPA last recommendation in 2003 approximately 26% of the published articles state the recovery efficiencies of the methods described, which vary grossly ranging from 13% to above 95% regardless of the method. All protocols are time consuming and the cost for each sample is considerable. A satisfactory method would have to be efficient, cheap and fast. The methods that have evolved over the last 35 years lack in all three features. Efficiency leaves much to be desired. The cost of equipment and lab consumables is prohibitive for routine laboratories of all but the most affluent countries. Time required for detection, given the urgency of management interventions when it concerns drinking water, is long. Based on the great improvements in technique that have been reached, research is required on novel concepts, in order to develop a method that can be cheap, fast and more effective. International organizations, governmental and non-governmental, in liaison with ISO (International Organization for Standardization) and IEC (International Electrotechnical Commission) should collaborate closely to this end and standardise such a method, based on expertise in parasitology, away from commercially oriented policies.

# Poster Presentations

## P3 ***Cryptosporidium* and *Giardia* contamination of the Danube Delta, Romania**

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The River Danube collects wastewaters and agricultural run-offs during its flow through Europe, from its origin in Germany to the emptying point into the Black Sea via the Danube Delta. It is thus a waterway of considerable importance, both for transport and also as a water source. In a broader research project that evaluates contamination of the Danube Delta in Romania with respect to microbiology and toxicology; previous findings indicated varying degrees of contamination of water and sediments with bacteria of fecal origin. Thus, further studies analyzing contamination with *Giardia* and *Cryptosporidium* were carried out. The frequency and level of protozoa contamination and fecal bacteria along with physical parameters (pH, turbidity) were compared in the study area. Using dedicated software the results were spatially analyzed and will be presented in the form of a thematic map.

# Poster Presentations

## P4 Indicators for the presence of *Cryptosporidium* and *Giardia* in water: facts and perspectives

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Because many pathogens are not easily detected, indicator organisms are a fundamental monitoring tools used to measure both changes in environmental water quality or conditions and the potential presence of hard-to-detect target pathogenic organisms (WHO, 2008). It is important to distinguish 1) the process indicator, the group of organisms that demonstrates the efficacy of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection; 2) the faecal indicator, the group of organisms that indicates the presence of faecal contamination, such as thermotolerant coliforms or *Escherichia coli*. Hence, they only infer that pathogens may be present; and 3) model indicator organisms, the group/or species indicative of pathogen presence and behaviour respectively, such as *E. coli* as an index for *Salmonella*, F-RNA coliphages as models of human enteric viruses (IWA, 2001). In particular, indicators for the presence of *Cryptosporidium* oocysts and *Giardia* cysts have been targeted by many researchers since they are very resistant to chemical disinfectants and testing directly for the *Cryptosporidium* and *Giardia* is costly and time consuming and may give a negative result if the numbers of the protozoa are low. We reviewed the publications related to *Cryptosporidium* and *Giardia* indicators and the results found are very heretogenic. No reliable tool to predict the presence/absence of these pathogenic protozoa. For the reasons mentioned above there is a great need to determine suitable indicator organisms to predict the presence of *Cryptosporidium* and *Giardia* in water. Furthermore, there is a great need for technological improvement, standardisation and automation of the detection technology. *Cryptosporidium* and *Giardia* can now be precisely quantified using immunofluorescence microscopy (ISO/FDIS 15553:2006(E)); species specific identification asks for PCR-based detection. All these advanced detection methods require a high degree of expertise and sophisticated technology. More simple concentration and molecular detection technology should be available for the routine use in drinking water quality examinations.

# Poster Presentations

P5

## **Inertial microfluidic separation and concentration of *Cryptosporidium* spp. oocysts and *Giardia duodenalis* cysts – Towards lab-on-a-chip technology**

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There are a number of hurdles faced in detecting *Cryptosporidium* oocysts and *Giardia* cysts on foods. The present study addresses the issues of interfering food particles and PCR inhibitors released during the physical elution of parasites from foods through shaking in buffer or other means. Inertial microfluidics can be utilized to manipulate particles of a desired size within a heterogeneous solution into focused streamlines. The objective of this project was to assess the potential of microfluidics in enhancing the separation of *Cryptosporidium* oocysts and *Giardia* cysts from food debris. Initial results demonstrated the ability of the *Cryptosporidium* specific L40 chip and *Giardia* specific L70 chip to focus, and thus concentrate, (oo)cysts from a 10ml elution buffer. A 10-fold concentration was achieved in which 45.87% (n=3) and 72.44% (n=3) of oocysts and cysts respectively were recovered. Subsequently, the ability to separate and concentrate *Giardia* cysts by a modified method integrating the L70 microfluidic chip was evaluated for 25 g lettuce samples spiked with 300, 150, 75, 37, 18 or 9 cysts. As a direct comparison, a duplicate set of samples were analyzed by a conventional centrifugation method. The limits of detection were defined as the lowest number of cysts in which a positive result was obtained greater than 50% of the time, as determined by fluorescence microscopy. The percent recoveries were determined from the samples spiked with 300 cysts. The numbers of background food particles in certain samples were analyzed using flow cytometry. Samples which underwent concentration by the L70 microfluidic chips contained 10X fewer background particles in comparison to the conventional method. There was, however, a slight reduction in cyst recovery with the chips, which recovered 68.39% (n=3) versus the 80.00% (n=3) recovery obtained from the conventional method. This slight loss was reflected in the limit of detection in which the conventional method was able to detect 18 cysts per sample while the L70 method was only able to detect 37 cysts per sample (3 trials). Newly designed versions of the chips with 10 parallel separation channels, will reduce this inherent loss of unfocused cysts during the concentration and separation process. These preliminary results demonstrate the potential of microfluidic methods in concentrating and separating cysts from food particles, and suggest that these methods may have the potential to improve detection.

# Poster Presentations

P6 **Occurrence of *Cryptosporidium* species by nested PCR in the drinking and environmental water samples of Samsun, Black Sea, Turkey**

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The aim of the present study was to investigate the prevalence of *Cryptosporidium* spp. by nested PCR in the water samples of Samsun province of Turkey in the Black Sea area. Seventy six water samples taken from 25 different stations including drinking and environmental water were collected and investigated for the detection of *Cryptosporidium* oocysts. Samsun is a port and most crowded city in the Black Sea coast of Turkey including of 25 different stations which have been selected as water collecting sites such as, Kurdun, Milic, Yesilirmak, Irmaksırtı, Gelemen, Selyeri, Kirazlık, Kızılırmak and River Mert in its Boroghs Terme, Carsamba, Bafra, Tekkekoy.

Water samples were collected in the spring of 2012 and in the autumn of 2013. They were concentrated with  $\text{Al}_2(\text{SO}_4)_3$  and then purified by sucrose-gradient centrifugation. Genomic DNA was isolated from the sucrose pellets and the nested PCR assays for the *Cryptosporidium* 18SrRNA gene were performed.

While none of the investigated drinking water samples was positive, 25 environmental water samples were positive using nested PCR assays (32.9%). The occurrence of *Cryptosporidium* in environmental water was not known in many regions of Turkey and this study will contribute to demonstrate the contamination of the water supplies in the Black Sea. The regular assessment of these parasites in environmental water supplies should be followed to better understanding of these pathogen threats.



# Poster Presentations

P7

## **Occurrence of *Cryptosporidium* spp. oocysts in low quality water and on vegetables irrigated with low quality water in Kumasi, Ghana**

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Protozoan parasites belonging to the genus *Cryptosporidium* are transmitted e.g. by food and water and may cause severe diarrhoea, dehydration, weight loss and malnutrition. Ingestion of 10 oocysts can lead to infection and pathogenic symptoms. Thus, to characterize *Cryptosporidium* spp. contamination level of river water, irrigation water and lettuce, 10L of water and 16 lettuce samples were collected four times in the period September – October 2013, with weekly intervals from six sample sites in and around Kumasi, Ghana. Oocysts were purified from water by sedimentation for 2 x 48 hours or pulsifying of lettuce followed by immunomagnetic separation and quantification by immunofluorescence microscopy, with sensitivities of 2 and 9%, respectively. Oocysts were stored approx. six weeks at 4C° before enumeration and approx. two weeks on slides at room temperature. Oocysts were subsequently washed off the slides and attempts to characterize *Cryptosporidium* spp. positive samples were done by PCR amplification and sequencing of the SSU rRNA, the HSP70 and the GP60 genes. *Cryptosporidium* oocysts were found in 75% of the water samples and on 43% of the lettuce with concentrations of 53 – 3,268 per 10L water and 11 – 118 oocysts per 15 g of lettuce. Positive water samples on one or more occasions were demonstrated in all water samples from both rivers and farm sites while all farms had positive lettuce samples on all occasions. Rainfall seemingly lowered the concentration of oocysts in water but not on lettuce. Molecular characterization of *Cryptosporidium* positive samples was unsuccessful, thus no conclusions can be drawn concerning sources of contamination. Nevertheless, the detection of high prevalence and concentration levels of *Cryptosporidium* oocysts on vegetables consumed raw and in water with direct contact to humans entails a potential risk of infection in humans. Implementation of preventive measures based on this study should be considered and actions taken accordingly.

# Poster Presentations

P8 **Pilot-scale evaluation of an innovative pulsed UV Light technology's efficacy: irradiation of raspberries experimentally contaminated reduces *Cryptosporidium parvum* infectivity in immunocompetent suckling mice.**

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Fresh produce is now recognized a leading cause of foodborne illness. *Cryptosporidium* species, detected both in irrigation waters and on produce, have been the etiological agents of recent fresh produce associated outbreaks. *Cryptosporidium* has been shown to be resistant to many disinfectants used for food and remain infective for weeks on stored produce. There is a need for evaluation of novel technologies to improve decontamination practices and minimising the risk of transmission. Pulsed UV-light is one such technology which does not affect human and environmental safety and has the potential to inactivate *Cryptosporidium* on surfaces of raw and/or minimally processed foods. Raspberries were chosen because they are a known source of a related human intestinal parasite, *Cyclospora cayetanensis*. Our study was aimed to evaluate efficacy of pulsed light irradiation against in vivo infectivity of *C. parvum* oocysts. To inoculate the raspberries, purchased from a local producer, five 10mL spots of inoculum ( $6 \times 10^7$  oocysts/mL, Nouzilly isolate) were deposited on the skin of 20 fruits. Raspberries were irradiated with 4 flashes with a fluence of  $1 \text{ J/cm}^2$  (i.e.  $4 \text{ J/cm}^2$  total fluence). The irradiation doses used did not affect quality characteristics of raspberries after treatment. After elution, oocysts were isolated from raspberries by IMS and bleached prior to infection to maximize their infectivity. Doses of oocysts were prepared by serial dilutions and administered orally to NMRI mice litters. Oocysts from non irradiated raspberries were used as positive control of infection. Seven days after infection, mice were killed and the number of oocysts in the entire small intestine was individually assessed by IFCM after incubation of oocysts with a specific FITC-conjugated monoclonal antibody. Three out of 12 and 12 out of 12 mice were found infected when 10 and 100 non irradiated oocysts were given, respectively. Four/12 and 2/12 mice were found infected when  $10^3$  and  $10^4$  irradiated oocysts were given, respectively. Intestinal oocyst counts were lower in animals infected with 1000 irradiated oocysts (range:  $92 \pm 144$ /small intestine) or 10000 irradiated oocysts ( $38 \pm 82$ /small intestine) than in animals inoculated with 100 non irradiated oocysts ( $35,785 \pm 66,221$ ) ( $p=0.008$ ). In the conditions used, pulsed UV irradiation achieved reductions of 2 and 3 log<sub>10</sub> for an inoculum of  $10^3$  and  $10^4$  oocysts, respectively. These reductions are comparable to reductions obtained with raspberries inoculated with *Salmonella* and/or *E. coli* O157:H7. Pulsed UV-light has the added benefit of a relatively short treatment time. This pilot-scale evaluation indicates that pulsed UV-light may be an effective mode of decontamination for raspberries. More studies need to be conducted in an industrial context in order to validate the applicability of this technology.

# Poster Presentations

P9

## Presence and risk from *Cryptosporidium* and *Giardia* in raw water and wastewater

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The large waterborne outbreaks of cryptosporidiosis in Östersund and Skellefteå in Sweden resulted in increased focus on risks for environmental transmission of intestinal parasites. The risk for exposure through drinking water is of concern and some drinking water treatment plants (DWTPs) more or less regularly sample their raw water for analysis of parasites.

Even so, recommendations for sampling of surface waters were in place already before the outbreaks, but generally conducted only by the larger DWTPs. This study summarizes the results from parasite analyses at seven Swedish surface water DWTPs during 2003–2008. Out of the 200 analyzed surface water/raw water samples, 4% (8/200) were positive for *Giardia* and 11.5% (23/200) were positive for *Cryptosporidium*. Three percent (6/200) were positive for both *Giardia* and *Cryptosporidium*. Complementary data such as rainfall, turbidity and indicator organisms were investigated in relation to presence/absence of *Giardia* and/or *Cryptosporidium*.

Wastewater outlets release cysts and oocysts to surface waters and may contribute to risks related to surface waters. Analyses of untreated wastewater in our previous studies have however mainly been conducted in order to acquire knowledge of the infection rate in the population connected to the wastewater treatment plant. During the outbreak in Östersund the concentration of oocysts was 2-3 orders of magnitude higher compared to samples collected before the outbreak.

Increased awareness of the potential presence of parasites as such probably results in measures that can mitigate risks for exposure to humans and animals. Results from analyses of surface waters and knowledge of the prevalence of infection in the population can further improve microbial risk assessments and aid in decisions that can lead to optimal treatment of drinking water.

# Poster Presentations

## P10 **Detection of *Cryptosporidium* and *Giardia* in water**

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*Cryptosporidium* spp. and *Giardia* spp. are protozoan parasites worldwide distributed, which represent potential risk in human and may cause severe gastrointestinal symptoms, especially species *Cryptosporidium parvum* and *Giardia intestinalis*. The subject of our interest is presence of pathogen protozoa in potable and surface waters, according to ISO 15553/2006. During the winter time (January-February 2014.), we analysed surface water (The Danube River) in the water supplying area nearby plant „Vinca“. Three times, we discovered the presence of *Giardia* cysts: first time 20/10L, the second 30/10L, and the third time the result was 15 cysts *Giardia* spp./10L. This data are significant, because samples were taken from the location which belongs to water supplying area. Our findings show that this locality is on the risk for isolated infections and potential outbreak if the treatment failures. Also, we found the *Giardia* cysts positive rate was comparable to that of fecal bacteriological indicators. Discovering the presence of fecal indicators suggests the possibility of presence *Giardia* contamination. Our regulative is insufficient regarding to this issue (presence of Protozoa in water) and we are not obliged to carry out the permanent examination, so it is only a pioneer achievement and proposal to continue.

# Poster Presentations

P11      **Development of an international standard for detection and enumeration of *Cryptosporidium* and *Giardia* on fresh leafy green vegetables and berry fruits**

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An International Standard (ISO DIS 1877) is currently under development, which specifies a method that is applicable for the detection and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts on fresh leafy green vegetables and berry fruits. The method is based on removal of (oo)cysts from the berry and vegetable surfaces by buffer elution, followed by immunomagnetic separation (IMS) and fluorescence microscopy. The standard is prescriptive, but allows flexibility of choice with regard to IMS and fluorescence staining reagents. The method will detect all species and genotypes / assemblages that are known to be pathogenic for humans and also others that are not. With suitable controls, it may also be applicable for the examination of other fresh produce types. Availability of a standard will facilitate comparability of analytical data, and harmonisation of monitoring and surveillance activities. Publication of the standard is planned for late 2014.

# Poster Presentations

## P12 **Transport and survival of *Cryptosporidium parvum* oocysts in soil columns following applications of raw and separated liquid slurry**

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The widespread waterborne pathogen *Cryptosporidium parvum* is frequently transmitted to humans via contaminated drinking and recreational water. Nearly all drinking water in Denmark is groundwater, which can be contaminated with oocysts e.g. from application of contaminated manure to the field. Oocysts transport to groundwater requires that the oocysts are transported through soil and bedrock to the water table. The purpose of this study was to determine the potential transport of the protozoan pathogen *C. parvum* through soil to land drains and, subsequently water sources in a laboratory setup using simulated rainfall and six 20 cm long replicate intact soil columns. Two types of contaminated slurry: raw slurry and the separated liquid fraction of the slurry were applied ten cm into the soil, which was subsequently irrigated once a week over a four-week period. *C. parvum* oocysts were detected in the leachates from soil columns to which *Cryptosporidium* positive slurry had been injected. Although recovery rates were low, regardless of slurry type, *C. parvum* oocysts were detected from all soil columns. Variations in the leachate patterns were recorded between soil columns added raw and liquid slurry respectively with significantly more oocysts in leachate from the latter. At the end of the study soil columns were destructively sampled to establish the location of remaining oocysts within the soil. Distribution within the soil was almost similar in all the soil columns, with the majority of oocysts found in the first section where the slurry was applied and with numbers decreasing with increasing depth.

# Poster Presentations

## P13 Detection and genetic characterization of *Giardia duodenalis* in rabbits from Italy

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Molecular analysis of *Giardia duodenalis* isolates has identified eight distinct genetic groups (i.e. assemblages A to H), which differ in their host distribution [1]. Assemblages A and B are mostly associated with human infections but they are also found in many other mammals. The genetic identity of *G. duodenalis* Assemblages has been thoroughly investigated in several animals, but few studies have addressed their occurrence in rabbits. A study conducted in Europe revealed a prevalence of 5.9% in these animals [2]. Some evidences have shown that rabbits harbor mainly the zoonotic Assemblage B [3]. The present study investigated the occurrence of the parasite in rabbits from central Italy (Abruzzo region), alongside the evaluation of these animals as potential zoonotic reservoirs. A total of 162 faecal samples from rabbits of different origins (i.e. 23 privately-owned, 5 from pet shops, 121 from rural farms, 13 from intensive farms) were screened for the presence of *G. duodenalis* using a sucrose gradient centrifugation assay. Protozoan cysts were detected in faecal samples from 4 rabbits (2.47%) coming from 4 rural farm which kept the animals in cages with no outdoor access. The animals showed no clinical signs. The isolates were subjected to a genetic characterization using the small subunit ribosomal DNA (SSU), the triose phosphate isomerase (tpi) and the  $\beta$ -giardin (bg) genes, which showed the presence of the Assemblage B. The infection rate observed is lower than that found from other areas in Europe [2]. Other than in humans, Assemblage B has also been detected in cattle, sheep, horses, dogs, and cats. Despite no sound evidence has supported the zoonotic transmission of this Assemblage between humans and animals, case control studies have showed that a contact with farm animals may be related to increasing infection rates of giardiasis in humans [4, 5]. Thus, rabbits can act as potential reservoirs of *G. duodenalis* cysts which are infectious to humans. More extensive studies in rabbits in different areas are needed to better characterize the transmission of giardiasis between human and rabbits and to assess its actual public health significance.

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# Poster Presentations

## P14 **Presence of *Cryptosporidium* and *Giardia* in cattle and wild boars living adjacent to a watershed in Sweden**

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The presence of *Cryptosporidium* oocysts in brooks emptying into a reserve water shed raised interest about the possible sources and risk for zoonotic transmission. A small herd of Highland cattle was grazing adjacent to the water shed and faeces were collected from fresh faecal pads of cows and calves at pasture in spring 2012. During the autumn-winter of 2012-2013, faeces were collected from the colon of 93 wild boars that had been shot in the area. Age of sampled animals was estimated and sex and place of shooting was recorded. Analysis was done by FITC-staining and microscopy after cleaning and concentration by saturated NaCl flotation (cattle) or sucrose-sodium flotation method (wild boars, to also detect *Giardia*). All cattle samples were *Cryptosporidium* negative. Of the wild boars, 13 (14%) were *Cryptosporidium* positive with oocyst outputs of 400-1200 OPG. Two were *Giardia* positive at 400-800 OPG, one of these was also *Cryptosporidium* positive. Five *Cryptosporidium* positive isolates were sent for 18S rRNA-PCR and sequencing, and two were successfully analyzed and identified as *C. scrofarum*. *Cryptosporidium* infection was found in all age categories, with a slight decrease with increasing age. There was no difference between males and females. *Cryptosporidium* positive animals were scattered throughout the area, with aggregates of 2-3 positive animals in three spots. The *Giardia* positive animals were both 3-4 months old and shot in the same location. This was the first time *Cryptosporidium* and *Giardia* was investigated and shown in wild boars in Sweden. *C. scrofarum* has previously been identified in a few domestic pigs in Sweden [1]. This was a first small investigation and results are not enough to determine whether there is a zoonotic potential in infected wild boars. Cattle are known hosts for *C. parvum* but *Cryptosporidium* infection was not shown at all indicating a low infection pressure.

### Reference:

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# Poster Presentations

## P15 *Cryptosporidium* and *Giardia* infections in the Norwegian muskox (*Ovibos moschatus*) population

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The Norwegian muskox (*Ovibos moschatus wardi*) population originated from various introductions from Greenland to Dovrefjell national park, around 60 years ago. The Dovrefjell muskox population has fluctuated considerably over the last decade, largely due to disease outbreaks, and the current population is around 200-300 animals. Baseline information on the prevalence and intensity of various intestinal parasites, including *Cryptosporidium* and *Giardia*, among different age classes of the Norwegian muskox population, was determined from 90 faecal samples, collected during June 2012, from matched animals. *Cryptosporidium* oocysts were detected in 13 samples (14% prevalence) and *Giardia* cysts were detected in 6 samples (7% prevalence); all positive samples were scored as +, with most having no more than a few parasites per sub-sample. At least one sample from each age group was positive for *Cryptosporidium* oocysts, but too few positive samples were identified to determine any agedistribution pattern. For *Giardia*, positive samples were identified in adults and yearlings. DNA isolated from *Cryptosporidium* oocysts from two samples was successfully amplified at the SSUrRNA gene. Sequence analysis revealed the samples to be *Cryptosporidium xiaoi*. For one *Giardia* sample, genotyping was successfully performed at both the  $\beta$ -giardin gene and the *gdh* gene; this sample was identified as Assemblage AI. These results are discussed in relation to the other intestinal parasites detected in these samples. Additionally, the potential that the muskox infections with *Cryptosporidium* and *Giardia* originate from other sympatric host species (sheep and reindeer, respectively) is discussed.

# Poster Presentations

P16

## ***Cryptosporidium* in European and North American voles**

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We undertook the molecular characterization of *Cryptosporidium* spp. infecting wild voles (Arvicolinae) on two continents: Europe and North America. European voles (n=152) included 129 common voles (*Microtus arvalis*) and 23 bank voles (*Myodes glareolus*) collected at nine localities in the Czech Republic in 2012. North American voles (n=417) included 338 meadow voles (*Microtus pennsylvanicus*), 46 woodland voles (*Microtus pinetorum*), and 133 southern red-backed voles (*Myodes gapperi*) from sites in North Dakota, Minnesota, and Tennessee in the United States. All European samples were examined for the presence of *Cryptosporidium* sp. using aniline-carbolmethyl violet staining and PCR targeting the small subunit (SSU) rRNA and actin genes. Samples from North America were examined using PCR targeting the SSU rRNA and actin genes. In Europe, *Cryptosporidium* sp. oocysts were detected in two common voles and two bank voles. A further 10 voles (four common voles and six bank voles) were positive by PCR. *Cryptosporidium* infection of European voles was not associated with diarrhea. In North America, *Cryptosporidium* was detected in 173 meadow voles, 22 woodland voles, and 15 southern red-backed voles. Phylogenetic analyses revealed 10 genotypes infecting North American voles, including four novel genotypes from bank and common voles. *Cryptosporidium* genotypes from European voles were not found in other rodents, including field and domestic mice, caught at the same locality. In North American voles, two closely related genotypes were also found in white-footed mice (*Peromyscus maniculatus*), and a third genotype was also found in an eastern chipmunk (*Tamias striatus*). Future studies will examine host specificity using experimental infection models. Outcomes from this research will inform our understanding of *Cryptosporidium* coevolution with hosts. This study was funded by the Ministry of Education, Youth and Sports of the Czech Republic (LH11061), the Grant Agency of University of South Bohemia (011/2013/Z), and the United States Department of Agriculture, National Research Initiative (2008-35102-19260).

# Poster Presentations

## P17 *Cryptosporidium* in North American ground and tree squirrels

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We undertook a molecular characterization of *Cryptosporidium* infecting seven squirrel species, representing the Marmotini (ground squirrel) and Sciurini (tree squirrel) tribes, of the family Sciuridae. Ground squirrels examined included black-tailed prairie dogs (*Cynomys ludovicianus*), 13-lined ground squirrels (*Ictidomys tridecemlineatus*), golden-mantled ground squirrels (*Callospermophilus lateralis*), and eastern chipmunks (*Tamias striatus*). Tree squirrels included eastern fox squirrels (*Sciurus niger*), eastern gray squirrels (*Sciurus carolinensis*), and American red squirrels (*Tamiasciurus hudsonicus*). Out of 416 samples examined; 174 (41.8%) were *Cryptosporidium* positive based on PCR analysis of the small subunit (SSU) rRNA gene. The prevalence ranged from 28.6% (2/7) in eastern fox squirrels to 68% (36/53) in 13-lined ground squirrels. Sequence and phylogenetic analyses of SSU rRNA and actin genes revealed the presence of *C. ubiquitum*, *C. parvum*, *C. andersoni*, and multiple wildlife associated genotypes in squirrel species. Mixed infections were detected in tree squirrels, and most involved *C. ubiquitum*, which was the most common *Cryptosporidium* species in tree squirrels. *Cryptosporidium parvum* was the only *Cryptosporidium* taxa detected in both squirrel tribes. Continuing work is addressing how factors such as host biology and ecology contribute to the differences in *Cryptosporidium* taxa infecting ground and tree squirrel tribes. This study was funded by the United States Department of Agriculture, National Research Initiative (2008-35102-19260).

# Poster Presentations

## P18 **Microsporidia, *Cryptosporidium* and *Giardia* infections in mountain gorilla (*Gorilla gorilla beringei*) in National Park Virunga Volcanoes, Rwanda**

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Infectious diseases pose one of the greatest threats to endangered species, and a risk of pathogen transmission from humans to wildlife has always been considered as a major concern of tourism. Increased anthropogenic impact on primate populations may result in general changes in communities of their parasites, and also in a direct exchange of parasites between humans and primates. Out of the total of 100 mountain gorillas (*Gorilla gorilla beringei*) from seven groups habituated either for tourism or for research in National Park Virunga Volcanoes, Rwanda, screened for the presence of intestinal protists using molecular diagnostics, 33 were positive for tested parasites. The most frequently detected parasites were *E. bieneusi* in 18 and *Encephalitozoon* spp. in 11 animals. *Cryptosporidium* sp. was identified in 4 cases and *Giardia intestinalis* in none of them. Nine distinct genotypes of *E. bieneusi* including genotypes EbpA, D, C, gorilla 2 and five novel genotypes gorilla 4–5. *Encephalitozoon cuniculi* genotype II was more prevalent (10 cases) compared to genotype I (1 case). *Cryptosporidium muris* (n=2) and *C. meleagridis* (n=2) were detected in great apes for the first time. The gorillas habituated for research were more parasitized (prevalence 35.0–55.6%) compared to tourism groups (prevalence 9.1–33.3%). This work was supported by grant from the Grant Agency of the Czech Republic (grant no. 206/09/0927), and by OPVK 2.3 project -Development of Scientific Team and Laboratory for Infectious Diseases Common to Humans and Great Apes (CZ.1.07/2.3.00/20.0300).

# Poster Presentations

P19 **Long-term monitoring of microsporidia, *Cryptosporidium* and *Giardia* infections in western lowland gorillas (*Gorilla gorilla gorilla*) in different stages of habituation in Dzanga Sangha Protected Areas, Central African Republic**

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Due to growing ecotourism and research purposes several groups of free-ranging great apes have been habituated to human presence. Humans can act as reservoir of pathogens for great apes and thus close contact with them can pose a risk to their health and vice versa. Eighty nine western lowland gorillas in different level of habituation from Dzanga-Sangha Protected Areas in the Central African Republic were sampled for the occurrence of *Cryptosporidium*, *Encephalitozoon* spp., *Enterocytozoon bieneusi* and *Giardia* sp. Sequence analyses determined 28 individuals positive for above mentioned infections. The most frequently detected parasites were *Encephalitozoon* spp. in 15 and *E. bieneusi* in 8 samples, respectively. *Giardia intestinalis* was identified in 4 samples and *Cryptosporidium* sp. in a single case. This is the first report of *Cryptosporidium bovis* infection in great apes. Besides previously described *E. bieneusi* genotype D, three novel genotypes (gorilla 1–3) were identified.

*Encephalitozoon cuniculi* genotype I was more prevalent (12 cases) compared to genotype II (3 cases). All four detected *G. intestinalis* belonged to Assemblage A. These results suggest potentially negative impact of habituation on selected pathogens which might occur as a result of the more frequent presence of humans in the vicinity of both gorillas under habituation and habituated gorillas, rather than as a consequence of the close contact with humans, which might be a more traditional assumption. The study was supported by the grants of Grant Agency of the Czech Republic (No. 206/09/0927) and OPVK 2.3 project (CZ.1.07/2.3.00/20.0300).

# Poster Presentations

## P20 *Cryptosporidium*, *Giardia* and microsporidia in Indonesian orangutans

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Orangutan numbers have decreased dramatically over the last century. Sumatran orangutans (*Pongo abelii*) are red-listed by IUCN as critically endangered, and Bornean orangutans (*Pongo pygmaeus*) are listed as endangered. In addition to habitat encroachment, trade, and hunting, opportunistic parasites pose one of the important threats to orangutan populations. In total, 66 out of 790 Sumatran and Bornean orangutans were positive for *Encephalitozoon*, *Enterocytozoon bieneusi*, *Cryptosporidium*, or *Giardia*. Of these, 44 animals were positive for microsporidia, including 27 animals infected with *Encephalitozoon cuniculi* genotype II, seven animals infected with *E. cuniculi* genotype III, nine animals infected with *Enterocytozoon bieneusi* genotype D, and one animal infected with *E. bieneusi* genotype B. *Cryptosporidium muris* was identified in 19 orangutans, and *C. parvum* and a *C. tyzzeri*-like sp. were each detected in one animal. *Giardia intestinalis* was detected in one animal. These parasites have been detected in orangutans for the first time. The authors would like to thank the State Ministry of Research and Technology (RISTEK) and the Directorate for General for Natural Conservation (PHKA) for their cooperation and for their permission to conduct research in Indonesia also would like to thanks to Orangutan Tropical Peatland Project: Outrop for cooperation with field work. The study was financially supported by the UMI - Saving of Pongidae Foundation: project "Parasites and Natural Antiparasitics in the Orang-utan" and partly by the Czech Academy of Sciences, Grant No. P505/11/1163.

# Poster Presentations

P21      **Study on the comparative prevalence of *Cryptosporidium* sps. in piglets, calves and HIV infected humans in the periphery of river basins of Kathmandu valley, Nepal**

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A cross sectional study was carried out to know the comparative prevalence of *Cryptosporidium* in calves, piglets and HIV infected patients in the River basins of Kathmandu valley. Fresh fecal samples were collected from the animals and modified Ziehl-Neelsen Staining technique after Modified Sheather concentration method with centrifugation was applied for the detection of the oocyst. A total of 21 piglets (42%), 16 calves (32%) and 9 humans (18%) were found to be positive for *Cryptosporidium* oocyst. Of the piglet samples, higher prevalence was seen in Manohara River (40.65%) than Bagmati River (44.44%). About 61.5% of the one month old piglets, 66.67% of the two months old piglets, 21.42% of three months old piglets and 18.18% of four months old were found to be positive. Female piglets (62%) were found to have higher prevalence than males (57.14%). However lethargic piglets (66.67%) had higher prevalence than diarrhoeal (57.74%) and asymptomatic (32.35%). Among calves, Manohara (58.33%) had higher prevalence than Bagmati (23.68%), and male calves (42.42%) had higher prevalence than female calves (11.76%). The parasite was mostly prevalent in 4-6 months and highest occurrence was seen in depressed calves than others. Among the human samples, the parasite was more prevalent in males (21.87%) than females (11.11%). Age group 0-20 years had highest (10%) prevalence. Furthermore, the human samples from Manohara (29.16%) had more prevalence than from Bagmati (7.69%). Thus, it was concluded that *Cryptosporidium* was prevalent in domestic animals and HIV/AIDS patients at that area.

# Poster Presentations

## P22 Molecular prevalence and analysis of potential risk factors for *Cryptosporidium* spp. infection in bovine calves in North Western Himalayan region, India

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A study was carried out to investigate the prevalence and to identify factors associated with risk of *Cryptosporidium* infection in bovine calves over the period of March 2012 to February 2013 from Jammu province, (North Western Himalayan region) India. Faecal samples from 684 bovine calves (432 cattle and 252 buffalo) were collected from 1 day to 6 months of age. An overall 48.39% (331/684) samples were found positive for *Cryptosporidium* spp. using mZN technique and nested PCR of 18S rRNA gene. Prevalence was significant ( $p < 0.05$ ,  $\chi^2 = 23.60$ ) between cattle (55%) and buffalo (36.11%) calves. Statistical analysis showed that infection rate were significantly ( $p < 0.05$ ,  $\chi^2 = 25.69$ ) higher in calves aged less than 1 month (59.64%) than in those of 3-6 months of age (35.52%). Infection rate was significantly ( $p < 0.05$ ,  $\chi^2 = 67.73$ ) higher in diarrhoeic (62.14%) than in non-diarrhoeic calves (30.03%). As per season significant prevalence (71.92%) was recorded in winter. Results of the analysis of factors potentially associated with the risk of *Cryptosporidium* spp. infection revealed that calves less than one month of age and winter season were strongly associated with cryptosporidia infection. Quantification of *Cryptosporidium* oocysts revealed high percentage (42.60%) of animals excreted moderate oocysts intensity (2+), where as diarrhoeic animals excreted more oocysts than non diarrhoeic. DNA sequencing of two samples revealed presence of *C. parvum* isolates and homology with other reported variants revealed one isolate to be closer to human isolates suggesting its zoonotic potential.

**Key Words:** *Cryptosporidium*, cattle and buffalo calves, mZN, nested PCR, India



# Poster Presentations

## P23 Characterization of *Cryptosporidium* excretion by adult goats around parturition in French goat flocks

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Little is known about *Cryptosporidium* infection in adult goats. An increase of both prevalence and level of excretion of *Cryptosporidium* in periparturient goats has been previously suggesting that adults can be a source of oocysts for the kids. But, while *Cryptosporidium* species are well-described in goat kids with *C. parvum* and *C. xiaoi* being identified, species infecting adult goats are nearly unknown. Only one species, *C. ubiquitum*, has been described in 7 and 11 adult goats in 2 herds in Peru and France, respectively. The aim of this study was to characterize *Cryptosporidium* oocysts excretion in adult goats around parturition and to identify species to evaluate the role of adults in the infection of the kids in French goat flocks. Individual faecal samples (15 g) were collected from 20 adult goats per flock during the 15 days prior to parturition in 31 farms. After cesium chloride concentration of the oocysts, samples were screened by an immunofluorescence assay (MERIFLUOR® *Cryptosporidium*/*Giardia*, Meridian Biosciences Inc) and level of excretion was determined (oocysts/gram of faeces, opg). Positive samples were withheld for molecular identification based on PCR on the SSU rRNA gene. 19 out of the 31 flocks screened had at least one positive goat (flock prevalence: 61.3%). Most of the positive flocks had only one positive goat (10 out of 19); on the contrary, one flock had 13 positive goats. 48 goats were found to be positive out of the 618 goats screened (individual prevalence 7.8%). The mean intensity of infection was 113 opg [range: 6-2140 opg] and only three goats excreted more than 300 opg. None of the positive samples could be amplified by PCR 18S probably due to the low level of excretion. The present results indicate that the role of adult goats in transmission of infection by *Cryptosporidium* to kids is probably weak regarding the low prevalence and level of excretion, suggesting that infected kids are directly the main source of contamination of the environment. However, the absence of molecular characterization of the isolates prevents us to definitely conclude on the role of adult goats as an initial source of infection at the beginning of the kidding season.

# Poster Presentations

P24 ***Giardia* /Giardiasis in lambs - High Prevalence in Roquefort Basin and Rhone Alps region. Zoonotic potential?**

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**Background and objective:** *Giardia duodenalis* are well-known enteric protozoa affecting a wide range of domestic and wild animals and also humans. While most infections with *Giardia duodenalis* are asymptomatic, giardiasis symptoms include foul smelling diarrhea, pale greasy stools, stomach cramps, gas, nausea, vomiting, bloating, weight loss, weakness and impaired growth, thus constituting a disease of economic concern. In Europe, prevalence surveys in lambs remain limited. Due to the potential public health hazard of waterborne and foodborne giardiasis, this work was aimed at obtaining information on the prevalence of giardiasis and the potential zoonotic role of *Giardia duodenalis* affecting lambs in 2 areas of France with a large number of lambs breeding farms.

**Methods:** For the period from February to May, 2012, 150 stool specimens from 10 farms were collected in the Bassin de Roquefort from 30-60 days old lambs. During the period from October to December, 2013, a total of 105 lamb's faecal specimens were collected from 7 farms in the Rhône-Alpes Region (Drôme, Ardèche and Isère). Faeces were randomly collected directly from the rectum of lambs. Microscopic examinations were performed on concentrated faeces using the Baillenger's method.

**Results:** In the Bassin de Roquefort, 9/10 farms had at least 1 infected animal. Contamination rates ranged from 6% to 55%. The overall prevalence of *G. duodenalis* infection in lambs is 23.3% (IC95% [16.8-30.9]). In Rhone Alps region, contamination rates ranged from 40% to 87%, the overall prevalence of infection is 60.95%.

**Conclusion and perspective:** High prevalence of giardiasis in these 2 regions represents a health threat for humans. Genotyping and subgenotyping studies of specimens from humans and animals living in the same households are in progress to provide better understanding of the transmission dynamics of *G. duodenalis*.

# Poster Presentations

## P25 Occurrence and molecular characterization of *Cryptosporidium* spp. in dogs in Henan Province, China

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Few data are available on the infection rate and molecular characterization of *Cryptosporidium* spp. in dogs in China. In this study, 770 fecal samples from 66 locations in Henan Province were examined. The average *Cryptosporidium* infection rate was found to be 3.8%, with the dogs in kennels having the highest rate of 7.0% (.2=14.82,  $P<0.01$ ). The infection rate was 8.0% in less than 90-day-old dogs, which was significantly higher than that in other age groups (1.1–3.8%; .2=18.82,  $P<0.01$ ). No association was noticed between the infection rate and the gender of dogs. Twenty-nine *Cryptosporidium*-positive samples were amplified using PCR at the small subunit rRNA (SSU rRNA), 70 kDa heat shock protein (HSP70), and actin loci. Sequence analyses of these amplicons identified only *Cryptosporidium canis*, which showed 100% identity to published sequences of the SSU rRNA, HSP70, and actin genes. Considering the large number of dogs in China and close contact between dogs and humans, the role of *C. canis* in the transmission of human cryptosporidiosis merits attention.

**Keywords:** Infection rate, Dogs, *Cryptosporidium canis*, SSU rRNA, HSP70, Actin

# Poster Presentations

## P26 Multilocus genotyping of *Giardia duodenalis* in dairy cattle in Henan, China

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**Background:** *Giardia duodenalis* is a common and widespread intestinal protozoan parasite of both humans and animals. Previous epidemiological and molecular studies have indicated *Giardia* infections in different animals and humans, but limit information about occurrence and genotypes of cattle giardiasis is available in China. In this study, the occurrence of *G. duodenalis* in dairy cattle, along with the potential and importance of zoonotic transmission, was assessed.

**Methodology/Principal Findings:** A total of 1,777 fecal specimens were randomly collected and examined from 15 dairy cattle farms in different cities of Henan province, China. 128 samples were positive for *G. duodenalis* infection by microscopy. All positive samples were genotyped based on 16S rRNA, bg, gdh and tpi genes. Seven new sequences of bg and four of gdh for *G. duodenalis* Assemblage E from cattle were found. Two genotypes of *G. duodenalis* were detected, namely Assemblage E (n=58), Assemblage A (n=21), with two mixed infection cases of Assemblage E and A. *G. duodenalis* subtypes were determined using the established nomenclature system based on multilocus sequence polymorphism (tpi, gdh and bg genes). Only 56 isolates were successfully amplified at all three loci. Nine novel multilocus genotypes (MLGs) E (MLGE1-E9) and two MLGs A (a novel subtype AI, previously detected subtype AII-1, with a mixture of subtype AI and AII) were found in the present study.

**Conclusions/Significance:** The present study provided preliminary data on prevalence and molecular characterization of *G. duodenalis* infection in Henan province. The same MLG in *G. duodenalis* derived from humans and cattle suggests that MLG AII-1 may be the main zoonotic subtype.

# Poster Presentations

## P27 *Cryptosporidium erinacei* n. sp. (Apicomplexa: Cryptosporidiidae) in hedgehogs

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The morphological, biological, and molecular characteristics of *Cryptosporidium* hedgehog genotype are described, and the species name *Cryptosporidium erinacei* n. sp. is proposed to reflect its specificity for hedgehogs under natural and experimental conditions. Oocysts of *C. erinacei* are morphologically indistinguishable from *C. parvum*, measuring 4.5–5.8 µm (mean=4.9 µm) × 4.0–4.8 µm (mean=4.4 µm) with a length to width ratio of 1.13 (1.02–1.35) (n=100). Oocysts of *C. erinacei* obtained from a naturally infected European hedgehog (*Erinaceus europaeus*) were infectious for naïve 8-week-old four-toed hedgehogs (*Atelerix albiventris*); the prepatent period was 4–5 days post infection (DPI) and the patent period was longer than 20 days. *Cryptosporidium erinacei* was not infectious for 8-week-old SCID and BALB/c mice (*Mus musculus*), Mongolian gerbils (*Meriones unguiculatus*), or golden hamsters (*Mesocricetus auratus*). Phylogenetic analyses based on small subunit rRNA, 60 kDa glycoprotein, actin, *Cryptosporidium* oocyst wall protein, thrombospondin-related adhesive protein of *Cryptosporidium*-1, and heat shock protein 70 gene sequences revealed that *Cryptosporidium erinacei* is genetically distinct from previously described *Cryptosporidium* species. This study was funded by projects of the Ministry of Education, Youth and Sports of the Czech Republic (LH11061) and the Grant Agency of University of South Bohemia (011/2013/Z).

# Poster Presentations

## P28 Epidemiological studies concerning cryptosporidiosis in calves in the center and northwest of Romania

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This study aimed to analyze the evolution of cryptosporidiosis in calves, following the prevalence, the influence of age, sex and seasonal variations and the identification of *Cryptosporidium* species implicated.

The research included 708 calves from 29 cattle farms in the center and northwest of Romania. The Henriksen stain was used for the highlight of *Cryptosporidium* spp. oocysts. Micro-measurements were conducted on 20 oocysts, with the use of Adobe Photoshop CS 4 software on images obtained with the Olympus BSX430 microscope. The Multiplex PCR technique using the gene Hsp 70 was employed in order to determine the *Cryptosporidium* species through molecular biology techniques. Specific primers were used for the genotypes: *Cryptosporidium parvum* (human genotype), *Cryptosporidium parvum* (bovine genotype), *Cryptosporidium canis*, and *Cryptosporidium felis*. We observed that 198 calves, 27.96% (95% CI=24.7%-31.5%), were eliminating *Cryptosporidium* spp. oocysts, starting with the age of 4 days with the highest prevalence between 2-3 weeks and the highest intensity between 1-2 weeks of age. The highest incidence of *Cryptosporidium* spp. infection was 70.21% at the end of winter and beginning of spring. It was detected one single type of oocyst measuring  $4.51 \pm 0.41 \mu\text{m}$  in length and  $4.07 \pm 0.33 \mu\text{m}$  in width, having a slightly oval shape. Based on the analysis of SSU rRNA gene of the 8 samples, 5 were positive for *Cryptosporidium parvum*, human genotype (62.5%) 3 were positive for the *Cryptosporidium parvum* bovine genotype (37.5%). The parasitological associations in calves between 0-8 weeks were: *Cryptosporidium* spp. and *Eimeria* spp. (6.8%), *Eimeria* spp. and *Strongyloides* spp. (0.7%), and in 3 calves the association between *Eimeria* spp., *Strongyloides* spp., and *Giardia* spp. was observed (0.42%). Oocysts elimination of *Cryptosporidium* spp. and *Eimeria* spp. was identified in 79.31%, respectively 68.96% of farms. This is the first systematic study concerning the epidemiology of cryptosporidiosis in calves in the center and northwest of Romania and demonstrates that this parasite is widespread in these regions.

# Poster Presentations

## P29 **Genotyping *Giardia lamblia* isolates from dogs in Guangdong, China based on multi-locus sequence typing**

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*Giardia lamblia* is one of the most common intestinal protozoan parasites, which is considered as a species complex, comprising at least eight distinct genetic groups, referred to as Assemblage A to H. To date, as the lack of high-resolution genotype identification based on one gene, a multi-locus sequence-typing scheme is needed on the molecular identification of zoonotic assemblages in dogs. Therefore, the aim of the present study was to genetically characterize isolates of *G. lamblia* from dogs in Guangdong, China using analysis of glutamate dehydrogenase (gdh), triose phosphate isomerase (tpi),  $\beta$ -giardin (bg) and small subunit ribosomal RNA (16S rRNA) loci combined. In the present work, a total of 216 dogs' faecal samples were collected in Guangdong, China. Four genes of tpi, gdh, bg and 16S rRNA from dog faecal samples were amplified separately by using normal or nested PCR. The phylogenetic trees were constructed with MEGA5.2 by using neighbor-joining method. Results showed that 9.7% (21/216) samples were found to be positive; moreover, ten samples were single infection (seven isolates Assemblage A, two isolates Assemblage C, and one isolate Assemblage D) and eleven samples were mixed infection where Assemblage A were the predominant, which was potentially zoonotic. These findings suggest that multi-locus sequence typing is more exact than single or double locus (loci) in genotyping, which provided a foundation on molecular identification and genetics of the *G. lamblia* in China. This work was supported by grant from National Natural Science Foundation of China (grant no. 31272551 and 30972179).

# Poster Presentations

P30

## **The prevalence, clinical impact and zoonotic significance of *Giardia* species in dogs and cats in Germany**

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*Giardia* is now the most common enteric parasite in well cared for dogs and cats in developed countries. This raises questions of the need for treatment in terms of the clinical effects of infection and potential impact on public health. With the former, it is still uncertain whether *Giardia* is always a clinical problem and if this varies between different breeds. It is also not known if the different species of *Giardia* to which dogs and cats are susceptible vary in virulence. Further, it is possible that concurrent infections with co-habiting protozoa such as *Cystoisospora* may exacerbate the consequences of *Giardia* infections. Since dogs and cats are susceptible to different species of *Giardia* which vary in zoonotic potential, and possibly their clinical impact, it is important to obtain data on the frequency of infection in urban areas. The few randomised surveys so far undertaken have reported contrasting results in the prevalence of the different species of *Giardia* in dogs and cats. The present study was undertaken to provide additional data on the prevalence of *Giardia* in cats and dogs in central Europe where previous studies with limited numbers of dogs have demonstrated differences in the frequency of zoonotic species in dogs.



# Poster Presentations

## P31 Tools for molecular detection of parasites

**Géraldine Leturnier<sup>1</sup>**, Marie Konarzewski<sup>1</sup> and Fabienne Loisy-Hamon<sup>1</sup>

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*Cryptosporidium* spp. and *Giardia* spp. are parasites responsible each year of millions cases of humans infection. Infection rates are from 1 to 10% in industrialized countries and much higher in developing countries from 10 to 30%.

Water is the main source of human contamination by these parasites both drinking or bathing water. Thus, it's not uncommon to find parasites in the water pool or sea, introduced by infected individuals, but also in food matrices like shellfish or vegetables. In addition, there are difficult to eliminate in the environment because they are protected by a very resistant shell (cyst or oocyst). Despite their persistence in time, the parasites are rarely present in high proportions in water or food matrices. As a result, their concentration and extraction of nucleic acids are difficult and timeconsuming steps. Several methods have been developed, including Methods 1622 and 1623 approved by the USEPA (U.S. Environmental Protection Agency) for water.

The methods for the diagnostic are currently based on immunofluorescence. They are tedious, poorly reproducible and difficult to implement by non-specialists. So we developed method eliminating all these constraints. For this, we chose the real-time PCR, a rapid, sensitive and reliable tool.

Primers and probes were defined following the same phases of validation performance tests including specificity, sensitivity and robustness for each of the targeted parasites. Firstly, the specificity of the primers and probes was developed in silico and demonstrated by experiment for each of the parasites. These tests have shown that no unwanted species is detected by couples. Then, the sensitivity of the pairs was compared on dilution series up 1eqG/L. This corresponds to the sensitivity required for our diagnostic test (1 oocyst or cyst, 5 eqG/reaction). Finally, the robustness tests were realized to certify the performance, both repeatability and reproducibility of the result. Moreover, the detection tests were optimized with the incorporation of an internal control. The internal control PCR ensures that the reaction was successful in all wells, and to demonstrate the presence or absence of inhibitors of PCR.

These new emerging pathogens necessitate to develop accessible and reproducible methods to be able to detect these parasites. The methods developed by the Ceeram for *Cryptosporidium* spp. and *Giardia* spp. meet the needs of simplicity, speed, specificity and sensitivity.

# Poster Presentations

P32 **Determination of in vitro effects of the treatment of trophozoites and cysts from the parasitic protozoan *Giardia duodenalis* with electrolyzed oxidizing water (EOW) with neutral pH**

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Giardiasis is a gastrointestinal disease of worldwide distribution caused by the parasitic protozoan *Giardia duodenalis*. This parasite has two stages, the vegetative form (trophozoite) and the infective (cyst) form and is a common cause of waterborne disease in which drug resistance is a reportedly growing issue. In most countries water treatment using sodium hypochlorite (NaOCl) is a common practice for disinfection, unfortunately an underestimation of NaOCl concentration will be harmless for trophozoites and cysts while an excess may be highly noxious for humans. Hence other antiseptic agents have been developed; among these are the so called electrolyzed oxidizing water (EOW), a wide-spectrum microbicide solution with neutral pH containing a mixture of diverse chlorine and oxygen active species. Herein we present the results of treating *G. duodenalis* trophozoites (susceptible and resistant clones to 250 µM of albendazole) or cysts with different concentrations of residual chlorine (0.106-0.007 mM) contained in serial dilutions of EOW (Esteripharma México, S.A. de C.V., pH 6.8). Exposure of trophozoites from albendazole-susceptible and resistant cultures and in vitro-derived cysts to EOW diminished the cell viability as assessed by the fluorescein diacetate-propidium iodide (FDA-PI) technique. On the other hand, when the same concentrations of EOW were applied to MDCK monolayers cell viability was affected only at the highest concentration of either EOW (0.106 mM residual chlorine) or commercial NaOCl solution as determined by trypan blue exclusion technique, suggesting a more selective activity of EOW against parasites. Interestingly, it was shown that EOW killed MDCK cells by apoptosis-like processes and NaOCl by necrosis as assessed by Annexin V-PI staining coupled to flow cytometry. These observations suggest that EOW would be a feasible preventing treatment for sources contaminated with *Giardia* and likely safer than NaOCl due to its pH closer to physiological conditions. Furthermore this solution does not produce cellular detritus associated with necrotic damage that in turn may induce undesired immune responses in the consumers.

# Poster Presentations

## P33 *Cryptosporidium* screening using Kinyoun technique in stray cats in Tehran, capital of IRAN

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*Cryptosporidium* is an intestinal protozoan parasite prevalent in a wide range of mammals. It has been recorded in many hosts including wild and domestic animals. Determining the infected animals could be a useful way to identify the potentially infective sources of the parasite. Forty stray cats, aged from 6 months to 13 years old from Tehran, capital of IRAN, examined in this survey. Collected fecal specimens were examined microscopically for the presence of acid-fast cryptosporidia in the Kinyoun stained smears. Among 40 tested animals oocyst shedding were found in 10 animals (25%) by the Kinyoun stain method. It is still unclear if a zoonotic transmission may occur between cats infected with *Cryptosporidium* and humans. Nevertheless stray cats are harboring the *Cryptosporidium* and should expect as a potential source of cryptosporidiosis in immunocompromised hosts.

# Poster Presentations

## P34 **Gone fishing! Capturing parasite nucleic acid sequences from different sources**

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*Giardia* and *Cryptosporidium* needs to be analysed on a molecular level to determine the species, subtypes or assemblies at hand. Sometimes the cysts are abundant in material to be analyzed and current protocols yield enough of reasonably pure material to meet the need. At times only a few oocytes are present and they may be embedded in a large amount of sample making isolation and subsequent analysis more difficult, if not impossible. To improve the capture and purity of parasite genetic material from sources containing only a few cysts and/or sources containing abundant other genetic material we have developed a method to capture the desired sequences by using specific hybridization probes coupled to biotin. The capture probes together with the hybridized target molecules are fished out by streptavidin coated magnetic beads. This method is highly specific, reasonably fast, applicable to both feces and water sources, yields very clean material for molecular analyses and can be performed within a working day, including PCR analysis. The working order is as follows. A larger volume of material (up to 15 ml) is treated at 100°C for 15 minutes to break the cysts and liberate the DNA. When the target sequence is captured a PCR is run to determine the efficiency of target capture and the level of inhibition. The results are compared with those from standard purification methods (i.e. Freeze&Thaw and purification by Qiagene Stool Kit). We have shown that fishing with capture probes works for both *Giardia* and *Cryptosporidium*. One of the major advantages is that this enables parallel molecular isolation and detection of parasites, bacteria and viruses from the same sample.

# Poster Presentations

## P35 **Analysis of the effect of the benzimidazole derivative CMC-20 on the cytoskeleton of *Giardia intestinalis***

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Metronidazole is the drug of choice for the treatment of giardiasis; however, drug resistance and treatment failure have been reported. Albendazole and nitazoxanide are alternatively used for giardiasis treatment and in this case, parasite experimental resistance has been induced for these two drugs. In an effort to have alternative drugs for the treatment of this disease, our group has synthesized a hybrid molecule of the benzimidazole with nitrothiazole, named CMC-20, that showed higher in vitro giardicidal activity than ABZ and MTZ. In addition, in a mouse model of giardiosis, CMC-20 reduced the time of infection as well as the number of cysts released in the feces of animals in comparison to the infection control. Proteomic studies performed with *G. intestinalis* trophozoites treated with CMC-20, demonstrated reduced expression of the cytoskeleton proteins, beta-tubulin, alpha-giardin and axoneme-associated protein. In order to characterize the effect of CMC-20 on the parasite cytoskeleton proteins, treated trophozoites were analyzed by immunofluorescence and western blotting. Immunofluorescence microscopy evidenced that CMC-20 induced morphological changes, a high number of rounded cells, similar to encysting cells and flagella retraction. However, no major distribution changes of alpha tubulin, beta tubulin, alpha-7.2 giardin and actin were detected. Interestingly, the median body was not detected in CMC-20 treated parasites. CWP-1 presence confirmed that CMC-20 induced the encystment process in some parasites and it co-localized with alpha-7.2 giardin in ESV and on the cyst surface. Western-blot analysis demonstrated that CMC-20 decreased the expression of alphetubulin, beta-tubulin and alpha-7.2 giardin but induced the expression of CWP-1. In conclusion, CMC-20 induced qualitative and quantitative changes on the cytoskeleton of *G. intestinalis* trophozoites and it is an encystation stimulus.

# Poster Presentations

P36 **Reactive oxygen species induced by albendazole causes DNA damage in *Giardia duodenalis* trophozoites leading to apoptotic-like death**

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The control of *Giardia duodenalis* infection is carried out mainly by drugs, among these albendazole (ABZ) is commonly used. The cytotoxic effect of ABZ involves binding to  $\beta$ -tubulin; however, it has been suggested that oxidative stress may also play a role in its parasitocidal mechanism. We analyzed the effect of ABZ in *Giardia* clones susceptible and resistant to different concentrations of this drug (1.35, 8 and 250  $\mu$ M). Reactive oxygen species (ROS) determined with DCFH-DA was higher in the ABZ-susceptible strains compared with the resistant one. Flow cytometry using DNA staining with PI showed that ABZ blocked cell cycle in susceptible clones while cells in S-phase diminished as parasites became resistant to ABZ. Likewise, ABZ induced the formation of 8-OH-dG groups and DNA degradation determined by electrophoretic analysis, which indicated nucleic acid oxidative damage. Furthermore, phosphorylation of histone H2A was detected in trophozoites treated with 250  $\mu$ M ABZ. Moreover, ABZ treatment resulted in PS exposure on the parasite surface, an event related to apoptosis. When cysteine, the major antioxidant in *Giardia* was added to trophozoite cultures exposed to ABZ, a protective effect on parasite growth was observed. On the other hand, lipid oxidation and protein carbonylation showed no significant differences. All together these data suggest that ROS induced by ABZ affects *Giardia* genetic material through oxidative stress mechanisms and induces apoptotic-like events.

# Poster Presentations

P37

## **Detection of anti-*Cryptosporidium* antibodies in human oral fluid using a multiplexed recombinant *Cryptosporidium* protein-coupled microsphere assay**

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Sero-prevalence studies suggest that *Cryptosporidium* exposure/infections are common and therefore indicate that stool testing under-estimates infection. Oral fluid is a potential alternative specimen to blood serum for evaluation of antibody responses to infection. Additionally, oral fluid antibodies to gastro-intestinal pathogens can be good markers of recent infection. The collection of oral fluid is non-invasive, simple, safe and painless and samples can be submitted directly from subject to laboratory by post. Sampler sponges such as Oracol (Malvern Medical Developments, Worcester, UK) readily collect crevicular fluid, which is rich in immunoglobulins. Previous preliminary work by US Environmental Protection Agency has shown that recombinant *Cryptosporidium* proteins-coupled to microspheres which have different spectral addresses can be used in multiplex to assay for anti-*Cryptosporidium* antibodies in human serum and oral fluid, using the Luminex-100 instrument. We have adapted and evaluated this method to utilise magnetic microspheres and the BioRad BioPlex-100 instrument. The method is being validated against a time series of serum and oral fluid samples from known cases of cryptosporidiosis for which we have previously gathered conventional western blotting data. Once validated, the Cryptosporidium Reference Unit (Swansea, UK) envisages using the assay to investigate the population extent of exposure, for example during outbreaks. In the first instance the CRU anticipates investigating a consenting cohort of veterinary students in the UK some of whom developed cryptosporidiosis following an animal handling class and a comparison group from a different faculty where there was no animal contact scheduled. This method has potential usefulness not only in the measurement of the extent of exposure to *Cryptosporidium* during outbreaks but also for monitoring interventions. It may also indicate infection where symptomatic illness has not occurred thereby revealing exposures otherwise not detected.

# Poster Presentations

**P38 Mucosal immunization of female mice with two recombinant antigens (SA35 and SA40) prior and during the gestation protects newborns from *Cryptosporidium parvum* infection.**

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*Cryptosporidium parvum* mainly affects the susceptible mammals in the early stages after the birth because their immune system is yet immature to trigger a specific response and the innate immunity is insufficient to contain the infection. For the same reason, an effective immunization strategy for young animals as well as for infants may be difficult as the immune system is yet undeveloped. However, the transfer of maternal antibodies to protect the neonate offspring from *C. parvum* infection would be considered to contrast cryptosporidiosis in the early weeks after the birth. In this study the ability of two recombinant antigens, namely SA35 and SA40, which represent immunogenic portions of two *C. parvum* secreted proteins Cpa135 and Gp900, to induce protection to *C. parvum* infection in BALB/c mice was assayed. SA35 and SA40 antigens were immunologically characterized by intraperitoneal administration in adult BALB/c mice. Both antigens singularly or in combination induced a high and persisting IgA and IgG responses in serum and a relevant IgA response at intestinal level. Splenocytes from immunized mice were able to proliferate in vitro when stimulated with a crude extract from *C. parvum*.

To test the ability of SA35 and SA40 to induce protection, three-days old neonate mice born from females immunized by mucosal exposure with the combination of the two peptides were infected with two different doses of *C. parvum* oocysts. The excreted oocysts as well as the intracellular forms of the parasite were counted 9 days after the infection, which is the time for the presumptive peak of the infection. In the group of newborn mice infected with the highest infective dose ( $10^5$  oocysts), the intracellular forms of *C. parvum* were counted by flow cytometry, showing a 40% of reduction. Otherwise, oocysts counted on the faecal pellet by immunofluorescent assay did not show significant reduction when compared with the control group. Remarkably, the group of mice infected with the lowest infective dose ( $4.5 \times 10^3$  oocysts) showed a reduction of 96% of all the *C. parvum* forms in the intestinal contents respect to the control group. To confirm this observation we performed a sensitive Real-Time PCR assay based on the COWP gene as genetic marker. The induction of a maternal immune response could be a promising way to protect the offspring from *C. parvum* infection.



# Poster Presentations

P39 **Drying of *Cryptosporidium* oocysts and *Giardia* cysts to slides abrogates use of vital dyes for viability staining**

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The use of vital dyes (particularly propidium iodide and DAPI) for assessing the viability of *Cryptosporidium* oocysts and *Giardia* cysts has provided useful information for a range of studies. Although the original articles describing the use of these methods stipulate staining in suspension, and this approach has been widely used, some recent studies have dried the samples to microscope slides prior to staining. As no formal comparison of dye exclusion/inclusion on dried parasite preparations and parasites in suspension has been published in the study described here we conducted such a comparison, with staining and enumeration by both methods conducted in two laboratories. Nine *Giardia* isolates and eight *Cryptosporidium* isolates were included in the study. The results demonstrate that drying samples to slides prior to staining gives a lower estimate of viability than when the parasites are stained in suspension.

# Poster Presentations

P40      **Long-term storage and real time PCR detection of *Cryptosporidium* from in vitro cultures**

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In vitro culture represents an important tool for diagnosis and characterisation of *Cryptosporidium* strains, allowing amplification of samples from relatively small amounts of starting material. The in vitro methodology developed by Hijjawi et al. [1] allowing culture of *Cryptosporidium* in monolayers of host cells, has great potential, but remains a research tool rather than a diagnostic method. Here we present methodological improvements in two areas allowing in vitro culture to be more widely adopted for diagnostic purposes. The first concerns storage of infected mammalian cells in liquid nitrogen and subsequent successful recovery of living *Cryptosporidium*; this allows long-term storage of the non-resistant thin walled oocysts which are predominantly formed when *Cryptosporidium* is cultured on monolayers. The second innovation concerns demonstration of *Cryptosporidium* in monolayers using real time PCR; this avoids uncertainty over the success of inoculation and subculturing, and reduces the time needed between isolation of oocysts from a sample and first characterization of the infection. Together these methodological developments improve the utility of the Hijjawi method for *Cryptosporidium* diagnosis and epidemiological research.

Reference:

[1] Hijjawi NS, Meloni BP, Morgan UM, Thompson RC, 2001. Complete development and long-term maintenance of *Cryptosporidium parvum* human and cattle genotypes in cell culture. Int J Parasitol. Aug; 31(10):1048-55.

# Poster Presentations

## P41 Human cryptosporidiosis in Belgrade, Serbia

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Cryptosporidiosis is caused by coccidian parasites of *Cryptosporidium* spp. Cryptosporidiosis in humans usually results in self-limited watery diarrhea in immunocompetent subjects but has far more devastating effects on immunocompromised patients. Cryptosporidiosis in humans is mainly caused by the zoonotic pathogen *Cryptosporidium parvum* and the anthroponotic pathogen *Cryptosporidium hominis*. The objective of this retrospective study was to analyze frequency of cryptosporidiosis cases diagnosed in Parasitological Laboratory of the Clinical Center of Serbia from January 2000 to December 2013. In patients with clinical suspicion of cryptosporidiosis (diarrhea in people who have weakened immune system or a high index of suspicion for cryptosporidiosis in immunocompetent individuals presenting with gastroenteritis) a minimum of three stool samples were requested. A modified Ziehl-Neelsen stain was used to detect cryptosporidial oocysts in airdried, methanol-fixed smears prepared directly from a fecal sample and after formalin-ether concentration technique. Occasionally the rapid immunochromatographic assay (RIDAQuick *Cryptosporidium*) for detection of *Cryptosporidium* antigens in stool samples was available (R-biopharm Diagnostic; Germany). Molecular diagnosis for *Cryptosporidium* was not available at the time of the study in Serbia. *Cryptosporidium* oocysts were found in the stools of 25 patients; maximum to 5 cases per year. Cryptosporidiosis was diagnosed in 17 AIDS patients, 3 renal transplant patients, 2 travelers with diarrhea after their returned from the African continent and, in December 2010, we notified a first family outbreak (three members) in Serbia with unclear the source of infection. A correct parasitological diagnosis of cryptosporidiosis requires a high index of clinical suspicion, adequate diagnostic method and repeatedly examination of samples. The rapid immunochromatographic test could be useful when oocysts of *C. parvum*/*C. hominis* species are very rare. Introduction of molecular methods for the detection of *Cryptosporidium* species could help in assessment of zoonotic capacity and in the selection of preventive measures.

# Poster Presentations

P42      **Infection status of *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*, and *Cyclospora cayetanensis* among diarrheal patients in the Republic of Korea during 2012-2013**

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Infection of *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*, and *Cyclospora cayetanensis* as water- and food-borne parasite was investigated in hospital-based diarrheal patients in the Republic of Korea, 2012 to 2013. A total of 5,598 stool samples were collected from all over the country. Protozoa was analyzed by age, gender and seasonal pattern. A total positivity of enteric protozoan infection was 6.96%, and 1.46%, 3.5%, 1.17%, and 0.82% were showed by *C. parvum*, *G. lamblia*, *E. histolytica*, and *C. cayetanensis*, respectively. In seasonally, the positivity was peaked in April to June and September to November, and it was showed that enteric protozoan infection mainly increased in the change of seasons. By gender, there was similar to state of infection rate. Interestingly, the positivity in less than 1-year-old group (10.01%) was highest among age groups, following 51-60-year-old group (7.59%) and over 60-yearold group (6.91%). Especially, *G. lamblia* (5.60%) was presented the main parasite in less than 1year-old group. Information on hospital-based protozoan in terms of gender, age, and seasonal patterns may improve diarrheal medical care, reduce the burden of acute gastrointestinal infections and help the development of control strateties for diarrheal diseases in the Republic of Korea

Keywords: *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, Republic of Korea

# Poster Presentations

P43

## Tools for Stools: identifying diagnostic needs for *Cryptosporidium*

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*Cryptosporidium* is an intracellular protozoan transmitted by ingestion of oocysts through water or food. Two species are thought to be the most common, the anthroponotic *Cryptosporidium hominis* and the zoonotic species, *Cryptosporidium parvum*. A variety of microscopy diagnostic procedures exist for *Cryptosporidium* as well as commercially available immunodiagnostic tests, several of which have been used in large epidemiological studies. The recent availability of molecular based tests for *Cryptosporidium* has enabled a lower threshold of oocyst/gram detection, but identified the need to better define the gold standard for *Cryptosporidium* diagnosis. In a recent convening, experts proposed a “gold standard framework” to capture the attributes needed for clinical and subclinical diagnosis of *Cryptosporidium*. Similarly, four diagnostic use scenarios - a test for clinical diarrhea and subclinical infection burden estimation, a test to evaluate treatment efficacy and a point of care diagnostic were identified as high priority needs for achieving our goals of reducing the global burden of *Cryptosporidium*. Target Product Profiles (TPPs) are in development for each of these use scenarios, and existing or novel, innovative technological approaches will be applied to these high priority diagnostic needs.

# Poster Presentations

P44      **A comparison study of different methods used in the detection of *Giardia lamblia* on fecal specimen of children**

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Objective: The purpose of this study was to compare results obtained using a single fecal specimen for O&P examination, direct immunofluorescent assay (DFA), and two conventional staining methods.

Design: Hundreded and fifty children fecal specimens were collected and examined by each method. The O&P and the DFA were used as the reference method.

Setting: The study was performed at the laboratory in the Basic Medical Science Institute JPMC Karachi.

Patients or other participants: The fecal specimens were collected from children with a suspected *Giardia lamblia* infection.

Main outcome measures: The amount of agreement and disagreement between methods. 1. Presence of giardiasis in our population. 2. The sensitivity and specificity of each method.

Results: There was 45 (30%) positive and 105 (70%) negative on DFA, 41 (27.4%) positive and 109 (72.6%) negative on iodine and 34 (22.6%) positive and 116 (77.4%) negative on saline method. The sensitivity and specificity of DFA in comparision to iodine were 92.2%, 92.7% respectively. The sensitivity and specificity of DFA in comparisoin to saline method were 91.2%, 87.9% respectively. The sensitivity of iodine method and saline method in comparison to DFA were 82.2%, 68.8% respectively. There is mark diffrence in sensitivity of DFA to conventional method.

Conclusion: The study supported findings of other investigators who concluded that DFA method have the greater sensitivity. The immunologic methods were more efficient and quicker than the conventional O&P method.

Abbreviations: DFA = direct immunofluorescent assay; O&P = ova and parasite

# Poster Presentations

## P45 *Cryptosporidium* – what's hidden in the reporting data?

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About 10% cryptosporidiosis cases in the UK are linked to reported outbreaks, which involve drinking water, recreational water use (especially at swimming pools), animal contact (such as at petting farms), food consumption and person to person spread (such in day care nurseries). The control of drinking waterborne cryptosporidiosis has resulted in a decline in the number of outbreaks linked to this exposure and a change in the characteristics of outbreaks: the average number of reported cases per outbreak has reduced in England and Wales. Between the introduction of structured outbreak surveillance in 1992 and the introduction of water quality regulations specifically directed at *Cryptosporidium* in 1999, there were 62 outbreaks of which 34% were linked to mains drinking water supplies. Between 2000 and 2012 there were 136 outbreaks and only 4% were linked to drinking water. Mains drinking water outbreaks usually involve more reported cases than those linked to other exposures. However, most cases are not diagnosed and the actual size of outbreaks is generally substantially larger than the numbers of cases reported. There is evidence from the detected outbreaks that even small ones fuel further cases in the community and therefore influence the overall burden of illness. We hypothesise that small, undetected outbreaks are occurring more frequently than is currently recognised and that these could be detected by enhancement of surveillance, informing preventative and control measures and ultimately a reduction in overall cases of cryptosporidiosis. Rolling analysis of two years of questionnaire data obtained from laboratory confirmed cases in one public health administrative unit in England, and the detection of spatiotemporal clusters using SaTScan software, identified outbreaks in excess of expectation based on national data. These outbreaks were small and associated with swimming pool use or, less commonly, direct contact with animals. The frequency of these outbreaks suggests that frequent small-scale transmission in swimming pools is an important contributor to disease burden. Identification of swimming pool level risk factors may inform disease control and preventative measures. The critical control points for infection control at swimming pool settings need to be identified and communicated to the swimming pool industry and users.

# Poster Presentations

## P46 Transmission routes for paediatric cryptosporidiosis and giardiasis in Chandigarh, Northern India

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Although giardiasis and cryptosporidiosis are known to be important causes of paediatric diarrhoeal disease, especially in settings or countries with sanitation challenges, the most important transmission routes in particular situations, and the factors that impact on those transmission routes, remain largely speculative. Potential transmission routes include direct hand-to-mouth, person-to-person or animal-to-person (zoonotic infection), or routes including a transmission vehicle (e.g. drinking water, recreational water, food); determining which transmission routes are of most relevance within a community enables targeted implementation of barriers to reduce the risk of infection. The Para-Clim-Chandigarh project is directed towards investigating the transmission of intestinal parasites (including *Cryptosporidium* and *Giardia*) in Chandigarh, Northern India with specific emphasis on foodborne and waterborne transmission and how this may be influenced by local weather patterns and projected climate changes. Chandigarh has well-defined seasons, and transmission pathways of these pathogens may change with the weather. Preliminary data collection will be conducted during February-April 2014 with selected sampling of water sources, irrigation water, local livestock and vegetables sold at local markets; where possible, sampling will be based on the history of exposure to children admitted to PGIMER with intestinal parasitoses. The results obtained, in conjunction with information from PGIMER records on hotspots for paediatric infection, will be used to determine more targeted sampling and investigation of contamination in conjunction with environmental variables, particularly those associated with weather and climate. In addition, how potential transmission vehicles become contaminated will be investigated. Later in the project, risk assessment and the effects of measures to reduce transmission will be investigated. Here we present the results of the preliminary data collection and analyses, along with information on the occurrence of paediatric cryptosporidiosis and giardiasis in Chandigarh.



**P47 Detection and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. in preschool children attending day care centers in Majadahonda, Madrid (Spain)**

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*Giardia duodenalis* and *Cryptosporidium* spp. are two of the most important diarrhoea-causing parasitic protozoa in humans. Both pathogens are transmitted through the faecal-oral route and are frequently associated to waterborne and foodborne outbreaks worldwide. Direct transmission person-to-person or animal-to-person is also possible. Children under 5-year-old are particularly susceptible to giardiasis/cryptosporidiosis. In Spain, little epidemiological and molecular information is available regarding the current status of *G. duodenalis* and *Cryptosporidium* spp. infections in children attending day-care facilities. Therefore, we aimed to estimate the infection rates of these parasites in apparently healthy preschool children attending day care centers in the town of Majadahonda (Madrid), and to identify factors associated with increased risks of giardiasis/cryptosporidiosis. Individual faecal samples were obtained from 90 child volunteer attending three day care centers. Collected samples were simultaneously tested for the presence of *G. duodenalis* and/or *Cryptosporidium* spp. by a variety of diagnostic assays including conventional and immunofluorescence microscopy, immunochromatography, and PCR. Genotyping of *Giardia* positive samples were carried out using a semi-nested *gdh* PCR and a nested  $\beta$ -giardin PCR. Restriction fragment length polymorphism (RFLP) analyses with *Nla*IV, *Rsa*I, and *Hae*III restriction enzymes were performed on aliquots of the products from all PCR amplifications. *Giardia* assemblages were confirmed by sequencing both strands of the DNA, and a phylogenetic analysis was conducted to investigate strain similarities among the obtained *Giardia* isolates. A socioepidemiological questionnaire was used to identify variables potentially associated to giardiasis/cryptosporidiosis in the children population under investigation. Overall, the prevalences of *G. duodenalis* and *Cryptosporidium* spp. were estimated at 15.5% and 4.4%, respectively. No mixed infections were detected. *G. duodenalis* and *Cryptosporidium* were found in 3/3 and 2/3 day care centers, respectively. By age group, 2-year-old toddlers were found to have the highest prevalence rates of giardiasis/cryptosporidiosis. No specific symptomatology was noted in the vast majority of the children analyzed. A total of eight *Giardia* isolates were characterized as Assemblage BIV, but attempt to genotype *Cryptosporidium* isolates failed. Although limited, our results seem to indicate that giardiasis and cryptosporidiosis are relatively frequent in under 5-year-old children in Central Spain, highlighting also the role of asymptomatic carriers in the dissemination of these diseases. These preliminary data should be confirmed in future studies with larger children populations from other Spanish geographical regions.

# Poster Presentations

## P48 Giardiasis in Cartagena de Indias (Colombia): an eco-epidemiology focus

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**Background:** Giardiasis is a common parasitosis caused by *Giardia intestinalis*, a cosmopolitan protozoan that infect humans as well as wild and domestic animals. Socio-economic aspects and habits are important factors for transmission. Ecological conditions can also influence transmission rates, but this aspect has not been studied in our environment so far.

**Objective:** To obtain a species distribution model of *Giardia intestinalis* and eco-epidemiological variables that influence transmission dynamics of giardiasis in Cartagena de Indias. **Methods:** Fecal samples of human and dogs were obtained in areas with high rates of acute diarrheal disease, and analyzed by Lugol staining to identify parasite cysts or trophozoites. Genotyping was done by PCR of the triose-phosphate isomerase gene. Data about socio-economical status were collected from the government information system. Geographical coordinates of sampling, socioeconomic variables and genotyping results were analyzed using QGIS 2.0.

**Results:** 111 out of 202 samples were positive for giardiasis. Genotyping showed Assemblage A in 5.1% and Assemblage B in 92.3% of human samples, respectively. Dog samples were typed as Assemblage B. Spatial distribution showed a focus of giardiasis in an area (Ciénaga de la Virgen) characterized by swamps with mangroves, low levels of public infrastructures and education, and low income.

**Conclusions:** Giardiasis in Cartagena is related to socio-economical aspects and ecological conditions that favor transmission, likely with a zoonotic component.

**P49 Retrospective multicenter evaluation of symptomatic cryptosporidiosis cases in French kidney-transplanted patients**

**Loïc Favennec**<sup>1</sup>, Nathalie Kapel<sup>2</sup>, Karine Guyot<sup>3</sup>, Emmanuel Dutoit<sup>4</sup>, Gilles Gargala<sup>1</sup> and French cryptosporidium network<sup>5</sup>

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Limited information is available about the incidence of human cryptosporidiosis in France where it is still considered a rare and not notifiable disease. The incidence of cryptosporidiosis in renal transplant recipients is presently poorly defined. Renal transplant recipients are a well defined population of patients who are carefully followed-up by nephrologists in association with GPs. The aim of this work was to analyse data recovered in 2012 by the French ANOFEL *Cryptosporidium* network to evaluate the incidence and characteristics of cryptosporidiosis in this particular situation. A french cohort of 27,484 kidney transplant recipients was followed. Twenty-nine cryptosporidiosis cases (i.e. 0.1%) were reported. Patients consisted of 17 males and 12 females, mean age 45 yrs, range 9 to 76 yrs, including 5 pediatric patients (3 males and 1 female, mean age 10.5 years (9-11)). Among 28 patients with clinical symptoms, all patients had diarrhea, it was noted nausea (6 patients), abdominal pain (13), fever (6), weight loss (14), dizziness (1), muscle pain (1), and fatigue (1). Throughout the course of the disease, all but one patient were hospitalized for profuse diarrhea and alteration of renal function. For 23 patients, duration of symptoms before parasitological diagnosis ranged from 1 to 77 days (median=9.5 days). In all patients, parasitological diagnosis was obtained by microscopically oocyst detection in feces. Association of *Cryptosporidium* with other intestinal parasites was not observed. Species molecular identification was performed in 25 patients, revealing *C. parvum*, *C. hominis*, *C. meleagridis* and *C. felis* in 13, 10, 1 and 1 patients, respectively. There was no difference of clinical symptoms between patients infected with either *C. parvum* or *C. hominis*. There was no difference of *Cryptosporidium* species between patients living in urban and rural areas (*C. hominis*: 7/9 and 2/9 patients, *C. parvum*: 7/13 and 6/13, respectively). Six cases (5 with *C. parvum*) were reported during the first 6 months and 23 cases during the last 6 months (8 *C. parvum*, 10 *C. hominis*, 1 *C. felis* and 1 *C. meleagridis*). A direct cause of contamination was suspected in for 6 patients: 4 were in close contact with a diarrheic patient, 1 with a parakeet, and 1 with contaminated food. Among the 19 documented cases, 6 patients attended swimming pool in the weeks before the onset of symptoms. Moreover, in most patients creatininemia increased throughout the course of cryptosporidiosis episode. Blood monitoring revealed an increase in tacrolimus blood concentration in infected patients. Cryptosporidiosis results in a significant increase in morbidity as well as a worse functional prognosis of the graft and testing for *Cryptosporidium* should be included in screening procedures for early microbiological diagnosis of diarrhea.

# Poster Presentations

## P50 **Investigation of the long term health sequelae following acute infection with *Cryptosporidium parvum*: an outbreak follow-up study**

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To investigate health sequelae (including irritable bowel syndrome, IBS) occurring after resolution of acute *C. parvum* infection in adults associated with a food-borne cryptosporidiosis outbreak in England, we undertook a longitudinal study. A total of 197 patients, resident in England, aged 16 years or over, diagnosed with *C. parvum* infection during May 2012 were asked to complete self-administered questionnaires - either using a secure online platform or paper format - at 6 and 12 months after diagnosis. Informed consent was obtained from all participants. A total of 54 participants were recruited to the study (recruitment rate=27%). Females were overrepresented among participants (74%) in comparison with potential participants (67%). There was no statistically significant difference in the mean age of those who did and did not take part ( $p=0.18$ ). 14 males and 36 females completed the 6-month follow-up questionnaire online (mean age 39.8 years). Having accessed the web-link, only 2 people failed to complete the questionnaire (completion rate 96%). A further 4 females completed the questionnaire in paper format (statistically significantly older mean age of 67.8 years). 39 of 54 recruited participants completed the 12-month follow-up questionnaire (retention rate=72%). Pre-existing IBS was reported by 11 people. The severity of acute cryptosporidiosis symptoms did not appear to be related to pre-existing irritable bowel syndrome: 55% of participants with and without IBS reported acute cryptosporidiosis symptoms as severe. At 6 months follow-up, 9 of 54 (17%) people reported symptoms which fulfil the Rome III criteria for diagnosis of IBS, 2 of whom did not report pre-existing IBS. At 12-month follow-up 44% of those with pre-existing IBS reported worsening of IBS symptoms in the 12 months after acute cryptosporidiosis. 26% of participants reported that they did not receive any information or advice about cryptosporidiosis. We conclude that a secure, online platform provides an efficient, accessible and acceptable method for personal health data collection from study participants. There is scope for improving communication between patients and professionals involved in the management of cryptosporidiosis. The significance of reported rates of IBS and IBS-consistent symptoms requires further research, and this study has informed the design of a Wales-wide prospective investigation of post-acute health sequelae, currently underway.

# Poster Presentations

P51 **Comparison of calves fed whole milk or milk replacer: impacts of experimental *Cryptosporidium parvum* infection**

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Enteric disease is a major health problem in calves, and diarrhea is associated with reduced weight gain and increased mortality rates in cattle production. Use of milk replacers with high protein content has been recommended to promote optimal health and growth rates in calves. However, whether health and growth is superior in calves being fed replacer compared to calves fed whole milk when exposed to pathogens is poorly understood. Cryptosporidiosis is known to affect the health and nutritional performance of calves. The aim of this study was to determine the influence of different milk feeding regimens on growth rates, infection dynamics and clinical disease in calves after experimental infection with *Cryptosporidium parvum*.

Calves between 2 and 7 days of age were divided into 4 groups (each of 6 calves), two which were fed daily with 8 L of milk replacer (26% protein/16% fat) and two which were fed daily with 8 L of whole milk. Two groups of calves, one on each diet, were experimentally infected with *C. parvum* oocysts. Faecal samples, blood samples, and clinical data were collected. The calves were enrolled in the study until weaning at approximately 8 weeks of age.

Preliminary results show that growth rates were not significantly different between the four groups. For the experimentally infected groups, oocyst excretion was higher in calves fed milk replacer compared with calves fed whole milk.

# Poster Presentations

## P52 **Effect of Enteric-parasitic Infection on Serum Trace Elements and Nutritional Status in Upper Egypt Children**

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Enteric parasitic infections still the cause of major health problems among Egyptian children as they have great morbid effect on their physical and cognitive development. Malnutrition makes children more prone to micronutrient deficiency which subsequently more vulnerable to parasitic infection. A vicious circle inside which, either parasitically infected or malnourished children are caught up in. The present study was to identify the effects of intestinal parasitism on micronutrient serum level (zinc and copper) and nutritional status among Egyptian children. A case control study was carried out on children aged from one to six years old who were attending the Assiut University Children Hospital outpatient clinic. After parasitological stool examination they were divided into Group1 (n=60) positive with enteric parasite and Group2 (n=60) non-parasitized, age and sex matched children. Anthropometric measurements were performed and expressed as weight for age (WFA), height for age (HFA), and weight for height (WFH) parameters. Serum Zinc (Zn) and Copper (Cu) were determined by Atomic Absorption spectrophotometer. The more commonly detected parasites were *Giardia lamblia* 28%, *Cryptosporidium* spp. 20%, and polyparasitism 18%. Analysis of the children's anthropometric measurements revealed that 63% of Group1 were malnourished, either in form of wasting or stunting or both aspects ( $P<0.001$ ). Stunting and wasting were more dominant among children infected with *G. lamblia* and *Cryptosporidium* spp. and most of them were below two years old. The *G. lamblia* and *Cryptosporidium* spp. patients had coincident decrease in serum Zn level and an increase of serum Cu. Parasites infection in children is common in our locality. *G. lamblia* and *Cryptosporidium* spp. were found to have a considerable impact on children nutritional status especially those who are below two years old. Linked micronutrient alteration was also documented. Targeting this circle and break it up in order to diminish the serious and lifelong health complications should be considered. Prevention and treatment of intestinal parasitic infection may have a positive impact on the micronutrient status and on the children general health at this important preschool age and by scale up Zn supplementation programs alongside enhancement of living, social and economic conditions.

**P53      Gene expression changes of *Giardia intestinalis* during in vitro interaction with host intestinal epithelial cells. The role of *Giardia* High Cysteine Protein Family**

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*Giardia intestinalis* colonizes the small intestine of humans and animals causing diarrheal disease known as giardiasis. This single celled eukaryotic parasite does not internalize into host cells but it rather attaches to the villi in the small intestine disrupting the proper functioning of the epithelial barrier. Here we have used an in vitro model of the parasite interaction with the host intestinal epithelial cells (IECs) to study by RNA-seq genome wide changes in gene expression from *Giardia* which might relate to persistence of infection and disease. Samples were collected at early time points of interaction (1.5, 3 and 4.5 hours) mimicking initial stages of infection. Between hundred to 200 genes presented altered mRNA levels. Among the highly up-regulated genes we found several members of the High Cysteine Protein (HCP) family, genes involved in cellular redox balance and genes from the lipid and nucleic acids metabolic pathways. In contrast, kinases, cell cycle and structural proteins were down-regulated, inferring a reduced cell proliferation. Hypothetical proteins were the major group detected as well in all three time points, indicating the vast number of important genes during infection which are still completely unknown. QPCR validated part of the RNA-seq and detected that the interaction media per se induced some of the changes observed which were attenuated when IECs were present. The HCP family consists of 86 members featuring high cysteine content in the form of CxxC/CxC motifs, resembling *Giardia* VSPs. In our RNA-seq, 20 to 24 members of this HCP family were highly up-regulated in the three time points analyzed and therefore we decided to further study this uncharacterized family and its involvement in parasite-host interaction. The cellular location of some members investigated by immunofluorescence of transfected *Giardia* cells with HA-tagged HCPs was found to peripheral vesicles and plasma membrane or to internal dotted structures somehow resembling parts of the ER. Interestingly, two HA-HCPs populations followed through several generations in normal growth conditions alternated their location between nuclei or nuclear periphery and PVs-plasma membrane. Further studies are ongoing. Additionally, proteomics studies of the interactions are also in progress in collaboration with Jonathan Wastling's group from Liverpool University, UK in order to understand the real status at the protein level of the changes observed by our RNA-seq.

# Poster Presentations

P54

## ***Giardia duodenalis* arginine deiminase: Sequence variation between isolates affects enzymatic activity**

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Depleting arginine is a frequent strategy of pathogens to modulate immune effector mechanisms and arginine metabolizing enzymes are considered virulence factors. Arginine deiminase (ADI) has been implicated in the virulence of *G. duodenalis*. We have recently shown that the response of human dendritic cells is altered when exposed to *G. duodenalis* ADI (Gd ADI) because of arginine depletion and formation of ammonium ions and citrulline. Conceptually, virulence factors are thought of as quantitative traits in pathogen populations. Here the hypothesis was tested that sequence variation detected between Gd ADI alleles affects functional parameters of the enzyme. Recombinant *G. duodenalis* ADI were produced based on encoding genes of sequenced isolates and the enzymes' K values were determined in vitro. In addition, K values of Gd ADI activities were determined in mmlysates of recent clinical parasite isolates and the respective adi genes were sequenced. Sequence variation correlated with changes in K values of the respective enzyme variants. Thus, our data add mfurther to the concept of Gd ADI being a molecularly defined virulence factor of these medically relevant protozoan parasites.



P55

## ***Giardia lamblia*: the effect of dietary zinc supplementation on growth performance, zinc status and immune response during the course of experimental disease**

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Several studies showed benefits of zinc supplementation on the incidence, severity, and duration of diarrhea; however the effects of this strategy may vary according to the etiological agent. Since *Giardia lamblia* (*G. lamblia*) is an important cause of parasitic diarrheal disease worldwide it was of interest to evaluate the effect of different levels of dietary Zn intake on growth performance, Zn status and immune response during infection. CD1 mice were fed 1 of 4 experimental diets: low Zn (ZnL), adequate Zn (ZnA), supplemented Zn (ZnS1) and highly supplemented Zn (ZnS2) diet containing 10, 30, 223 and 1383 mg Zn/kg respectively. After a 10-day feeding period, mice were orally inoculated with  $5 \times 10^6$  *G. lamblia* trophozoites and followed on the assigned diet during the course of infection (30-days). Body weight gain was recorded and blood samples were collected throughout the study. Diets and serum zinc levels were assessed by atomic absorption spectrophotometry; serum anti-*G. lamblia* IgG was measured by ELISA. *Giardia*-free mice fed ZnL or ZnA diet were able to maintain normal growth while *Giardia*-infected mice presented significant growth retardation and lower serum Zn levels. ZnS1 and ZnS2 diets avoided this weight loss and improved serum Zn levels despite the ongoing infection. Within 10 days following immunization, the mice in all four dietary groups responded by producing significant levels of specific IgG antibodies. Greater IgG antibody response was observed in the ZnS1 and ZnS2 groups when compared to the ZnL or ZnA ones. It appears that zinc supplementation triggers a higher production of specific IgG and so suggests that zinc was effective in up-regulating the host's immune response against this parasite. In conclusion, *Giardia lamblia* impaired growth performance in mice fed ZnL and ZnA diets. Zinc supplementation avoided this weight loss due to infection and improved the growth rate, even when compared to *Giardia*-free mice fed ZnA diet. Maximum growth rate and antibody mediated response were attained in mice fed ZnS1 diet with no further increases by feeding higher Zn levels (ZnS2). These findings reflect biological effect of Zn that could be of public health importance in endemic areas of infection. (Partially published, *Nutrients* 2013, 5(9), 3447-3460)

# Poster Presentations

P56

## Host cell interactions of a novel *Cryptosporidium* protein containing a C-type lectin domain

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*Cryptosporidium* is an Apicomplexan parasite that causes severe diarrheal disease worldwide, especially in immunocompromised individuals such as AIDS patients and malnourished children. However, the molecular mechanisms and proteins underlying *Cryptosporidium*-host cell interactions are not well understood. Proteins containing C-type lectin domains are known to mediate diverse cell interactions in organisms ranging from humans to viruses. Interestingly, proteins with C-type lectin domains have not been reported in apicomplexans. We previously identified a gene encoding a novel *C. parvum* mucin-like glycoprotein, CpClec, which is also predicted to contain a C-type lectin domain. The primary and tertiary structure of the CpClec C-type lectin domain is highly similar to those of mammalian C-type lectin receptors that mediate cell-cell interactions. Antibodies to CpClec recognize a ~120 kDa protein, much larger than its predicted size, suggesting significant posttranslational modification. CpClec localizes to the surface, dense granules and apical region of invasive stages of the parasite, as well as to dense granules and the feeder organelle of intracellular stages. To evaluate binding to host cells, a construct lacking sequences encoding the transmembrane domain and cytoplasmic tail was PCR amplified and cloned into the mouse IgG Fc-tagged mammalian expression vector pFUSE mlgG2a. Constructs were transfected into HEK 293T, CHO K1 and CHO Lec2 cell lines and purified by Protein G affinity chromatography. Binding of purified recombinant protein to Caco-2 and Hct-8 cells was assessed by fluorescence microscopy and immunoaffinity pull-down assays. Fc-tagged recombinant CpClec binds both Caco-2 and Hct-8 cells in a strong and specific, punctate manner compared to the Fc only control. Pull-down and SDS-PAGE analysis of Hct-8 cell lysates show a clear difference in proteins bound by Fc-tagged CpClec compared to Fc alone. Future studies are aimed at identifying the ligands bound by CpClec and evaluating the importance of these interactions during *Cryptosporidium* infection in vitro.

# Poster Presentations

**P57 Identification a signaling pathway of Binding immunoglobulin protein, BiP as a mouse dendritic cell maturation protein in *Giardia lamblia***

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Much remains unknown about the mammalian immune response to *Giardia lamblia*, a protozoan pathogen that causes diarrheal outbreaks. We fractionated protein extracts of *G. lamblia* trophozoites by Viva-spin centrifugation, DEAE ion exchange, and gel filtration chromatography. Resultant fractions were screened for antigenic molecules by western blots analysis using anti-*G. lamblia* antibodies (Abs), resulting in identification of *G. lamblia* binding immunoglobulin protein (GBiP). Immunofluorescence assay (IFA) of *G. lamblia* trophozoites using anti-GBiP Abs demonstrated that GBiP is localized in the endoplasmic reticulum (ER) of this protozoan. Intracellular amount of GBiP in *G. lamblia* did not vary during encystation. Maturation of mouse dendritic cells (DCs) in response to recombinant GBiP (rGBiP) was detected by increased expression of surface molecules such as CD80, CD86, and MHC class II; these mature DCs, produced pro-inflammatory cytokines (TNF- $\alpha$ , IL-12, and IL-6). rGBiP-induced DC-activation was initiated by TLR4 in a MyD88-dependent way and occurred through activation of p38 and ERK1/2 MAPKs as well as increased activity of NF- $\kappa$ B and AP-1. Moreover, CD4<sup>+</sup> T-cells stimulated with rGBiP-treated DCs produced high levels of IL-2 to induce T-cell proliferation. Together, our results, suggest that GBiP contributes to maturation of DCs to the immune response against *G. lamblia*.

# Poster Presentations

## P58 Investigating human humoral and cellular immune responses against recombinant *Giardia* antigens

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*Giardia lamblia* is a protozoan parasite that infects animals and humans worldwide. It is one of the most common protozoan infections in the world. A crude vaccine that prevents cyst shedding has been developed for cats and dogs, but no vaccine is available for humans. The immune responses towards this parasite have mainly been studied in mice, but studies in humans are few. Acquired immunity plays an important role in these responses as some studies have shown a reduction in susceptibility after an initial infection. However, further delineating the features of the immune response is necessary to understand the reasons for the variable symptomatology and development of a protective vaccine. Our aim is to investigate the humoral and cellular responses against selected recombinant antigens cloned from the GS\M strain of *G. lamblia*.

Multiple surface and metabolic antigens have been identified using mass spectrometry. These proteins combined with known immunodominant proteins found in earlier studies give us a pool of potential candidates for our investigational research. Nine different proteins have been selected based on their potential as antigenic targets against *G. lamblia*. Using DNA from the Assemblage B strain of *Giardia*, we are cloning the proteins using a pET-15b vector in an *E. coli* model. Three of these have been successfully cloned. The protein produced from the modified clones will be purified using Ni-Nta resin beads and dissolved in PBS and glycerol.

Blood samples for serum and peripheral blood mononuclear cells are obtained from *Giardia* infected patients and from a control group of persons with a low risk of ever having had giardiasis. Humoral immune responses are investigated by ELISA and western blots. Using flow-cytometry we evaluate antigen specific cellular immunity with activation and proliferation assays. This experiment will hopefully help us to better understand the variable host responses towards a *Giardia* infection. This may in turn clarify the differences in symptomatology and chronicity of the infections in humans. Further investigation will also help us to identify suitable antigens for subsequent vaccine experiments. Data from this work will be presented at the conference.

# Poster Presentations

P59

## Responses of cell lines to the real and simulated inoculations with *Cryptosporidium muris* oocysts

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In present study two types of cell lines, HCT8 and HT29, were used for an in vitro cultivation of the gastric parasite *Cryptosporidium muris*. Our observations were carried out after 24, 48 and 72 DPI using combined approach of light, electron and confocal laser scanning microscopy. So far, we succeed to detect free sporozoites with typically prolonged apical ends that seemed to search for appropriate host cell/infection site, few structures closely resembling full or already emptied cryptosporidian parasitophorous sacs, and free merozoites especially in HT29 cell line. Evidently, invading sporozoites preferred newly formed round cells or gaps in a discontinuous layer characteristic for young cell cultures. Interestingly, however, unexcysted oocysts of *C. muris* were found to be completely or partially enveloped by projections of individual host cells after 24 DPI in both cell lines. The experimental inoculation with polystyrene microspheres was designed in order to verify whether this behaviour of cell lines is provoked by oocysts of *C. muris* or it represents their innate reaction to foreign objects in general. Direct comparison and evaluation of both cell lines inoculated either with *C. muris* oocysts or with polystyrene microspheres confirmed that the enclosing of oocysts by HT29 and HCT8 cells was induced by the parasite. The microspheres were found only occasionally to be covered by a tiny filamentous projections arising from host cells or remnants from old cell cultures. Based on present data, we consider this to be a natural adherence of biological garbage to the surface of polystyrene microspheres.

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# Poster Presentations

## P60 Natural History of Cryptosporidiosis in the First Two Years of Life in Bangladesh

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*Cryptosporidium* spp. have recently been identified as a leading cause of moderate-to-severe diarrhea in infants worldwide [1]. The only FDA approved therapy, nitazoxanide, is not appropriate for young children, and there is no available vaccine. Human immunity to *Cryptosporidium* spp. is largely thought to be T-cell mediated [2-4], however acquired immunity to this parasite has not been defined. Additionally, the role of humoral immunity has not been established, though our group has demonstrated the protective effect of maternal breast milk IgA on breastfeeding infants in Bangladesh [5]. In this follow up of a community-based prospective cohort study, we aim to describe the natural history of *Cryptosporidium* spp. infection and identify correlates of mucosal and humoral immunity (serum and stool antibodies) in slum-dwelling Bangladeshi children [6]. Children were enrolled at birth and followed for two years, with active surveillance for diarrheal illness. Diarrheal and surveillance stool samples were tested for *Cryptosporidium* spp. using real time qPCR [7]. Enzyme-linked immunosorbent assay was used to test for serum anti-cryptosporidium IgG and fecal anti-cryptosporidium IgA. Anthropometric measurements were taken every 3 months. We followed 392 children from birth to age two. By age two, almost 80% of children in the cohort had been infected with *Cryptosporidium* spp. Asymptomatic infection (affected 75% of children) was more common than diarrheal infection (25% of children). Higher parasite burden, as measured by quantitative real time PCR, was associated with diarrhea rather than asymptomatic infection (T-test,  $p < 0.0001$ ). Using multivariable regression analysis, we found that children with asymptomatic *Cryptosporidium* spp. infection during the first two years of life were significantly more likely to have growth stunting at age two, when compared to children who were never infected ( $p = 0.035$ ). Positive anti-Cryptosporidium serum IgG at 12 months of age was associated with lower risk of *Cryptosporidium* spp. infection during the second year of life (log-rank test,  $p = 0.033$ ), however no protective effect was seen with positive anti-Cryptosporidium IgA in stool. History of *Cryptosporidium* spp. infection in year one did not predict risk of infection in year two. We also found that over 90% of samples tested were of *C. hominis* subtype, which is consistent with previous reports.

In summary, the burden of *Cryptosporidium* spp. infection in Bangladeshi children is largely subclinical, however is associated with significant growth faltering. This is the first study to demonstrate that *Cryptosporidium* spp. infection associated with diarrhea is related to higher parasite burden. Our findings suggest that human immunity to *Cryptosporidium* spp. may be acquired, which has important implications for the potential for future vaccine development.

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# Poster Presentations

## P61 Mitosomes behavior during the life cycle of the pathogenic protozoan *Giardia intestinalis*

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The mitosome is a double-membrane bounded organelle found in few unicellular eukaryotes, including the human intestinal parasitic protozoan *Giardia intestinalis* that also lacks mitochondria and peroxisomes and has been considered to be among the earliest branching eukaryotes. This flagellated protozoan grows in vitro as trophozoites and under some conditions differentiates into cysts, characterized by the absence of flagella, a rounded shape and the presence of a cyst wall. Using antibodies that recognize two proteins present in the mitosome, the heat-shock protein 70 (mitHSP70) and the giardial chaperonin 60 (giCpn60), we analyzed the presence and distribution of the mitosomes during the cell cycle as well as during the process of trophozoite-cyst transformation by confocal laser scanning microscopy and Western blotting. Our observations show that at early stages of the differentiation process (around 12hrs) there is a significant decrease in the extent of labeling of the cells and the number of mitosomes, which almost disappear after 21hrs, followed by recovery in the cyst stage. This information was confirmed by mRNA expression analysis thus indicating a process of modulation of formation of mitosomes during the life cycle of *G. intestinalis*. Electron microscopy tomography allowed the three dimensional reconstruction of mitosomes revealing the presence of rounded as well as elongated organelles.

Supported by: CNPq, FAPERJ, AUSU, PRONEX

# Poster Presentations

P62      **Adherence of *Giardia duodenalis* on epithelial cells is dependent on phosphorylation and dephosphorylation events**

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*Giardia duodenalis* is a parasitic protozoan that causes diarrhea and other symptoms which together constitute a disease known as giardiasis. While the disease process has been well defined, the mechanisms involved during interaction of the protozoan with the intestinal epithelium have not been fully elucidated. In this work we analyzed if phosphorylation/dephosphorylation at tyrosine, serine and threonine residues play some role on the adhesion of trophozoites to intestinal epithelial cells in vitro. Using antibodies recognizing phosphorylated residues we observed labeling at the parasite adhesion sites for serine and tyrosine. However, labeling for threonine was observed only in parasites. PI3Kinase seems to play an important role in the adhesion process. When kinase and phosphatase inhibitors (genistein, staurosporin, wortmannin and okadaic acid) were added to the interaction medium decrease of the number of trophozoites adhered to Caco-2 cells surface was observed, suggesting that a balance between phosphorylation and dephosphorylation appears to be crucial for parasites to reach a best performance to adhere. The present study provides further evidence regarding the in vitro interactions between intestinal cells and *Giardia* and contributes to a better understanding of the pathogenesis of giardiasis.

Supported by: CNPq, FAPERJ, PRONEX, UFRJ



# Poster Presentations

P63

## **Study of the role of the VP9B10A surface protein from *Giardia duodenalis* in the interaction with epithelial cells**

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Variable Surface Proteins are responsible of antigenic variation in *Giardia*, in experimental models these are important on the induction of protective response and have been associated with persistent infection of this parasite. To determine whether these proteins have other functions we selected the VSP910A and analyzed its role on the attachment of trophozoites to epithelial cells. This was approached by transfection WB trophozoites to obtain stable expression of this protein and by analysis of co-cultures with epithelial cells. Proteomic analysis of supernatants from cultures of *G. duodenalis* with monolayers showed that this protein was secreted during this interaction and it had proteolytic activity as determined by zymograms. Immunofluorescence and RT-PCR assays showed that the VSP9B10A protein and transcript were overexpressed in the WB trophozoites upon interaction with Caco-2/TC7. With trophozoites transfected a correlation between VSP9B10A protein release into the culture medium with the loss of integrity of the monolayers was observed, as well as F-actin contraction and tight junctions proteins Claudin-1, Occludin and ZO-1 relocation. All together these results provide evidence for a novel role for the VSP9B10A.

# Poster Presentations

## P64 **Development of a multilocus fragment typing tool for genotyping cattle-derived *Cryptosporidium parvum***

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Subtyping of *Cryptosporidium parvum* by single locus sequencing of the GP60 gene has been widely adopted internationally and as such provides a useful, readily portable library typing tool. However, more discriminatory typing tools are required in outbreak situations or to answer local epidemiological research questions, for example transmission dynamics of *C. parvum* within and between cattle farms. Multilocus fragment typing (MLFT), which uses length polymorphisms in micro- or minisatellite regions to differentiate genotypes, is rapid, cost-effective and can identify mixed genotypes. However technical difficulties arise when comparing results between laboratories as observed sizes depend on length of the fragments amplified, sequence composition, machine used and running conditions. Perhaps for these reasons there is currently no standard scheme.

*C. parvum* samples from both epidemiologically related, and unrelated, populations of cattle were used to trial an MLFT scheme. A panel of 6 markers was selected based on previous studies: MM5, MM18, MM19, TP14, MS1 and MS9. In addition a region of the GP60 gene was sequenced to provide GP60 subtype, which was added to the allelic profile to differentiate multilocus genotypes (MLGs). Fluorescently labelled PCR products were subjected to fragment analysis using capillary electrophoresis (ABI 3730). Trace files were then analysed using both Peak Scanner (Applied Biosystems) and STRand. The tool was assessed for performance in terms of typeability, specificity, discriminatory ability, repeatability and reproducibility. Nested primers improved typeability, which was acceptable at 87%. The primers appear to be specific as no amplification occurred when isolates of the common cattle-related non-*parvum* species were tested (*C. bovis*, *C. ryanae* and *C. andersoni*). Discriminatory ability was good (SID 0.92), especially when compared to GP60 sequencing alone. In the samples studied, two markers were monoallelic (MS1 and MS9) and may not be useful in future studies in cattle. In terms of repeatability, between-run variation was observed, however within-run variation was minimal, therefore allele calling was straightforward when representatives of all known alleles were included in each PCR plate. To measure reproducibility, some interlab comparisons have taken place and whilst some differences in reported fragment sizes were recorded, allele assignment was reproducible.

# Poster Presentations

## P65 A new set of primers for subtyping of *Cryptosporidium meleagridis* at the gp60 locus

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*Cryptosporidium meleagridis* is a common cause of cryptosporidiosis in avian hosts and the third most important species for human cryptosporidiosis, affecting both immunocompetent and immunocompromised patients. According to a recent report it has also been identified in cattle, highlighting the wide host range of this parasite.

DNA sequencing of the highly polymorphic 60 kDa glycoprotein (gp60) gene is a frequently used tool for evaluation of genetic diversity and transmission dynamics of *Cryptosporidium*. It is mainly used for subtyping of *C. hominis* and *C. parvum*, but other species, including *C. meleagridis*, have also been investigated using this locus. Genetic variation has been identified among *C. meleagridis* isolates from humans and birds and both anthroponotic and zoonotic transmission routes have been suggested. However, as quite few studies have included gp60 subtyping of *C. meleagridis* isolates, the amount of published sequences is limited. One explanation could be that current primer design is mainly based on *C. hominis* and *C. parvum* sequences, potentially limiting successful amplification of the *C. meleagridis* gp60 gene.

The aim of this study was to design primers suitable for gp60 subtyping of *C. meleagridis*. Initially ~1440 bp of the gp60 fragment of two *C. meleagridis* isolates were amplified using primers flanking the ORF of *C. parvum*. The obtained sequence data (~1,250 bp) were used to design primers for a nested PCR targeting *C. meleagridis*. Twenty isolates (16 from human and 4 from poultry) previously identified as *C. meleagridis* at the rRNA gene locus were investigated. Amplicons of the expected size (~1,000 bp) were obtained from all 20 isolates. Subsequent sequence analysis identified 3 allele families and 10 different subtypes. The most common allele family, IIIb, was identified in 12 isolates, represented by 6 subtypes, 4 new and 2 previously reported. Allele family IIIe was found in 3 isolates represented by 3 different subtypes, none of them reported before. Finally the same IIIg subtype, IIIgA31G3R1 was found in one human isolate and the four poultry isolates, all originating from a previously reported *C. meleagridis* outbreak at an organic farm.

Earlier attempts at our laboratory to amplify and sequence the gp60 gene fragment from the *C. meleagridis* isolates included in this study failed. Hopefully this new set of primers will encourage other researchers to include gp60 subtyping of *C. meleagridis* in their studies.

# Poster Presentations

P66

## Analysis of a single *G. duodenalis* cyst: Micromanipulation and DNA extraction

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*Giardia duodenalis* is a protozoan of worldwide distribution responsible for causing enteric infections in a wide variety of mammals, including humans. A considerable amount of data has shown that *G. duodenalis* should be considered as a species complex that comprises at least eight distinct genetic groups (assemblages A to H). Two phenomena have been frequent in molecular studies: (i) occurrence of heterogeneous gene sequences, characterized by the presence of polymorphic sites and (ii) divergence between loci when different assemblages are marked by distinct genetic markers used in the same sample. Allelic sequence heterozygosis, genetic recombination and mixed infections are the explanations for such events; however, only the analysis of a single cyst can demonstrate the existence of genetic recombination in the parasite. In the present study eleven cysts of *G. duodenalis* were isolated by micromanipulation technique (fig. 1), submitted to DNA extraction and subsequent nested PCR assay. For DNA extraction was used 5 µL of TE and 1 µL of proteinase K in each tube containing a single cyst of *G. duodenalis* and then the tubes were submitted to incubation cycles of 56°C for 2 hour and 95°C for 15 minutes, respectively. DNA amplification by nested PCR technique was evaluated using primers directed to β-giardin gene. The DNA extraction technique occurred with success in the individualized cysts of *G. duodenalis*, whereas was possible to observe, through agarose gel electrophoresis, DNA fragments of 511 bp approximately in six of eleven cysts isolated (fig. 2). Finally, the assays described in the present study can be used to studying the genetic variation of *G. duodenalis*, helping to clarify the epidemiology of infection as well as the taxonomy of this genus.

# Poster Presentations

P67

## Exploitation of genomic data to design novel detection targets for human infective *Cryptosporidium* and *Giardia*

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*Cryptosporidium* and *Giardia* are the most prevalent waterborne protozoan parasites associated with gastrointestinal diseases in human. As for other pathogens, good detection techniques are crucial for timely diagnosis and appropriate disease management. While traditional and molecular detection methods exist for both parasites, we sought to uncover novel targets allowing differential detection of human infective species and assemblages as well as strain typing to facilitate outbreak investigation and source tracking. We used published genome sequences for *C. parvum*, *C. hominis* and *C. muris* to identify putative species specific genes for the most prevalent human infective *Cryptosporidium* species (*C. parvum* and *C. hominis*). Similarly, comparative genomic analysis of *Giardia* assemblages A, B and E, enabled to identify genes that are potentially specific to Assemblage A and B. All putative specific genes were tested by PCR using reference strains (Iowa, Moredun and TU502 for *Cryptosporidium* and GS and WB for *Giardia*), in addition to well-characterised clinical strains. Specificity was further assessed by testing other *Cryptosporidium* species as well as nonhuman infective *Giardia* assemblages (C-F). Our results were similar for *Cryptosporidium* and *Giardia* and showed that the majority of the genes were not specific to any one Assemblage or *Cryptosporidium* species. Nevertheless, specificity was experimentally confirmed for few important genes of unknown function, which provide obvious utility as diagnostic targets. These key loci also show sufficient variation between lineages and strains to have utility in effective genotyping and warrant inclusion in future typing schemes. Functional characterisation of these genes is likely to improve our understanding of the evolution and host adaptation of *Cryptosporidium* and *Giardia*.

# Poster Presentations

P68      **Genetic characterization of *Cryptosporidium parvum* isolates from calves and humans in High Normandy-France: IlaA22G3R1 found in calves.**

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To apprehend the transmission dynamics of cryptosporidial infections and to investigate diversity of *Cryptosporidium parvum* isolates from humans and calves and its potential role as a source for human cryptosporidiosis in High Normandy region–France (the first region for dairy cattle breeding in France), 22 *Cryptosporidium parvum* isolates from sporadic human cases of cryptosporidiosis in the region and 38 isolates from diarrheic calves were subtyped by sequence analysis of the 60 kDa glycoprotein (gp60) gene.

The most common subtype was IlaA15G2G1, identified in 81.66% of isolates (19/22 in human samples and 30/38 in calves). IlaA17G2R1 were also encountered in 3.33% in both human and calves. In human IldA23G1 (1/22) was also recorded. In calves, IldA17G1 (n=1) and *Cryptosporidium* Ila (IlaA16G1R1 (n=1), IlaA20G1R1 (n=2), IlaA18G2R1 (n=1), IlaA22G3R1(n=1)) were detected. The subtypes IlaA22G3R1 had not been diagnosed elsewhere in calves until this study. The study demonstrates that IlaA1G2R1 *C. parvum* infection was common and widespread in humans in this region and that calves have a high prevalence of *C. parvum* IlaA15G2R1. The presence of *C. parvum* subtype IlaA15G2R1 indicates that calves from this region should be considered as a potential source of zoonotic *Cryptosporidium* oocysts. To go further, our ongoing study is aiming at investigating genetic structure and heterogeneity within the dominant gp60 subtype found in high Normandy using multilocus sequence typing (MLST) at 12 genetic loci.

# Poster Presentations

## P69 Genotyping of *Giardia duodenalis* in human feces by assemblage-specific PCR assay

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*Giardia duodenalis* (syn: *G. Intestinalis*; *G. lamblia*) is an enteric protozoan that causes giardiasis in humans and in a wide range of domestic and wild animals. Studies based on genetic analyses have been showing that *G. duodenalis* can be assigned to at least eight different assemblages (A to H) in which only the assemblages A and B have been considered potentially zoonotic. Currently, studies on molecular characterization of *G. duodenalis* are being carried out through the use of assemblage-specific primers. In the present study assemblage-specific primers directed to *tpi*, *gdh* and *orfC4* genes which were previously designed for Real time assay were used to amplify DNAs of assemblages A and B of *G. duodenalis* by conventional PCR assay. Ten stool samples from human origin were used in the analysis and in seven of them both assemblages were simultaneously observed by at least one gene. The results can be explained by true mixed infection or genetic recombination, however, the only way to evaluate the real participation of these phenomena would be through the analysis of a single *Giardia* cyst. Moreover the present findings bring out the importance of developing methods for cyst isolation to evaluate the sexual recombination in *G. duodenalis*.

# Poster Presentations

## P70      **Genotyping of *Giardia lamblia* human isolates based on triosephosphate isomerase (TPI) gene by PCR-RFLP in Iran**

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Giardiasis is an infection of the digestive system caused by tiny parasites called *Giardia lamblia* (also known as *Giardia intestinalis* or *Giardia duodenalis*). *Giardia* sp. is the most common intestinal parasite of humans and other animals throughout the world. Infection with *Giardia lamblia* most often results from fecal-oral transmission or ingestion of contaminated water. Diarrhea is the most common symptom of giardiasis, other symptoms can include abdominal cramps, bloating and flatulence. Most patients are asymptomatic and most infections are self-limited. Isolates of *Giardia lamblia* are classified into seven assemblages, based on the characteristics of the glutamate dehydrogenase (GDH), small-subunit (SSU) rRNA and triosephosphate isomerase (TPI) genes. Assemblages A and B infect humans and a broad range of other hosts. The purpose of this study was to genotype of human isolates of *Giardia lamblia* by PCR in Karaj City. 60 positive fecal samples of *Giardia lamblia* were collected. DNA extraction and amplification of TPI gene by nested-PCR successfully were conducted. All samples were positive. To determine genetic differences sequencing on 5 samples were conducted. Alignment of the TPI sequences obtained with reference sequences indicates the presence of 2 genotypes of *Giardia lamblia* (A and B). The results of RFLP technique show that 29 of 50 (58%) isolates belonged to Assemblage A and 19 of 50 (38%) belonged to Assemblage B and 2 samples were not determined. Thus, the Assemblage A might be dominant genotype in Karaj city.



# Poster Presentations

P71

## ***Giardia lamblia* Assemblage B: A predominant genotype among children hospitalized for acute diarrhoea at a Paediatric Hospital in Accra, Ghana**

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Giardiasis remains a common gastroenteritis in many developing countries including Ghana. The disease which is caused by a protozoan parasite, *Giardia lamblia* continues to spread among children as result of inadequate supply of treated water, poor sanitation and unhygienic practices. Being a zoonotic disease, it is suspected that, some human infections could be as a result of presence of infected domestic and farm animals in our homes. Although the recent application of molecular tools in epidemiological studies has helped to understand how the disease spreads among humans, animals, and the environment, very little information is available on the genotyping and transmission routes of *G. lamblia* in Ghana. In the present study, isolates of *G. lamblia* from Ghanaian children in Accra were genotyped to determine the predominant assemblages. The study was a prospective cross-sectional hospital-based, conducted in Accra, Ghana. A total of 485 patients comprising of 365 diarrhoeic and 120 non-diarrhoeic children of age=5 years, were studied. Stool samples were collected and tested microscopically, and by enzyme immunoassay kits. Positive samples were tested by the semi-nested polymerase chain reaction (PCR) and subsequently characterized into genotypes by PCR-RFLP, and nucleotide sequence analysis at the glutamate dehydrogenase, *gdh* gene locus. Demographic and clinical data were obtained by a structured questionnaire. A prevalence rate of 5.8% giardiasis was observed, and the prevalence in diarrhoeic children was significantly higher than non-diarrhoeic children ( $P<0.001$ ). Sex of child ( $OR=1.9$ ,  $95\%CI=0.715-4.982$ ;  $P=0.193$ ) and age ( $OR=2.8$ ,  $95\%CI=0.432-2.854$ ;  $P=0.729$ ) were both not risk factors associated with infection. *G. lamblia* Assemblage (genotype) B was the only genotype which was identified as a cause of infection among both diarrhoeic and non-diarrhoeic children. The presence of genotype B as the only prevailing genotype of *G. lamblia* indicates that infections from animals will be uncommon. The presence of infection among non-diarrhoeic children is of much concern, as it demonstrates the possibility of spread of infections unknowingly.

**Keywords:** *Giardia lamblia*; Ghana; Paediatric infection; acute diarrhoea; genotyping; risk factor

# Poster Presentations

## P72 High Diversity of *C. parvum* subgenotypes identified in dairy calves compared to beef calves : 1st report of *C parvum* IIaA22G3R1 infection in a dairy calf.

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*Cryptosporidium* is one of the most common non-viral causes of diarrhoeal disease in neonatal calves and one of the organisms of greatest interest because of its zoonotic potential. *C. parvum* represents the main zoonotic species and calves are considered a major host reservoir. Studies on molecular characterization of *C. parvum* have been mostly conducted in dairy calves. Little is known about subgenotypes infecting beef calves. In the present study, we undertook a molecular characterization of *C. parvum* in dairy and beef calves. Sampling was conducted: in (i) the Saône et Loire department for beef calves; the heartland of the Charolais, the pride of French meat trade and (ii) in Normandy region for dairy calves, the heartland of French dairy products. *Cryptosporidium* spp. oocysts were detected in stained smears, and positive faecal samples were further analyzed for molecular typing. Species identification based on the small-subunit rRNA and subtyping using the gp60 gene were carried out. *Cryptosporidium* positive samples were collected from 43 beef calves in Saône et Loire department and 38 from dairy calves in Normandy region. *C. parvum* species was exclusively identified. Sequence analysis of the gp60 gene showed IIaA15G2R1 as the most common subgenotype found, and was identified in 93.02% (40/43) of beef calves and 79% (30/38) of dairy calves. In beef calves, *C. parvum* belonging to subgenotypes IIaA14G2R1 (2/43) and IIaA17G2R1 (1/43) were also identified. Compared to beef calves, our findings highlighted the presence of high diversity of *C. parvum* in dairy calves; 7 alleles, belonging to the IIa family (6) and the IId family (1) were identified. Among them, the most common allele IIaA15G2R1 (30/38); IIaA20G1R1 (2/38); IIaA17G2R1 (2/38); IIaA16G1R1 (1/38); IIaA18G2R1 (1/38); IIaA22G3R1 (1/38) and IIdA17G1 (1/38). The subgenotype IIaA22G3R1 had not been diagnosed elsewhere in calves until this study. It was only identified in 2 cases of cryptosporidiosis in HIV+ patients (New South Wales, Australia). The lack of knowledge about this subgenotype suggests further investigations. Since allele IIaA15G2R1, which is strongly implicated in zoonotic transmission was identified; to track potential transmission of the parasite from calves to humans, *C. parvum* isolates from pediatric patients who attended to the Pediatrics of Rouen University Hospital were analyzed. Four different *C. parvum* subtypes were identified within the 23 human isolates; IIaA15G2R1 (19/23), IIaA17G2R1 (2/23), IIaA18G1R1 (1/23) and IIdA23G1 (1/23). Our data suggests that calves represent a potential source for human cryptosporidiosis in Normandy. Further genotyping and microsatellite analysis is ongoing.

P73 **Multilocus genotyping of *Giardia duodenalis* isolates from humans and cattle from Slovenia**

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Giardiasis is considered a potentially zoonotic disease, but the role of animals in human disease transmission needs to be firmly established. Due to the lack of morphological variation among isolates of *G. duodenalis* from different hosts, molecular biology techniques are increasingly used to estimate the genetic variability of this parasite and to improve the understanding of its epidemiology. In the present study, isolates from symptomatic patients with giardiasis from Slovenia were analysed by PCR and sequencing of three genetic loci, the  $\beta$ -giardin (bg), the triosephosphate isomerase (tpi), and the glutamate dehydrogenase (gdh). In addition, isolates from animals (cattle, sheep, and goats) were typed at bg locus by a PCR-RFLP assay. Out of 85 human isolates, 36 (42.4%) were successfully amplified and sequenced at all loci, and the results showed that 21 (58.3%) were typed as Assemblage A and 15 (41.7%) as Assemblage B. At the sub-Assemblage level, all Assemblage A isolates were All. High nucleotide variation was found among Assemblage B isolates, therefore subtyping was difficult to achieve. Indeed, allelic sequence heterozygosity was detected in 40% of the bg and tpi sequences and in 53.3% of the gdh sequences from Assemblage B isolates. Altogether, 9 of the 15 (60%) Assemblage B isolates had heterogeneous positions in at least one gene. Using PCR-RFLP, 35 out of 84 (41.7%) animal isolates could be typed, and all were Assemblage E. This was further confirmed by a new real-time PCR assay based on Assemblage E specific primers. The fact that only Assemblage E was found in animals suggests a minor role of cattle, sheep and goats in the transmission of giardiasis in Slovenia. Moreover, the finding that human isolates were of Assemblage B and of the anthroponotic sub-Assemblage All, supports human-to-human transmission as the most likely route of transmission of giardiasis in Slovenia.

# Poster Presentations

P74

## A shift in *Cryptosporidium* spp. subtypes in The Netherlands, 2012-2013

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Following the summer of 2012, a marked increase in cryptosporidiosis was observed in the Netherlands. An outbreak investigation in the form of a case-control study was performed to identify the source of this increase. In addition, *Cryptosporidium* spp. genotyping based on GP60 sequencing was also performed. The findings of the study did not reveal a common source for the outbreak. However, results of the genotyping found that 81% of cases were *C. hominis* type IbA10G2. This prompted us to begin a two-year population based case-control study in 2013, into the cause of sporadic cases of cryptosporidiosis. This study combines both data from the epidemiological survey and typing of *Cryptosporidium* spp. positive samples. The study is in collaboration with the National Institute for Public Health and the Environment (RIVM) and 17 regional microbiology laboratories that send in feces or fecal derived DNA of patients with *Cryptosporidium*. In the Netherlands, the general practitioner (GP) of a patient who presents with gastrointestinal complaints may request the patient sends a stool sample for testing. If a sample is identified as *Cryptosporidium* spp. positive, the GP is notified and sends an invitation to the case requesting participation in this study. The RIVM will determine the species, *C. parvum* or *C. hominis*, using a duplex real time PCR. Subsets of samples are genotyped using GP60 PCR, followed by sequencing and subsequent analysis. In 2013, the reporting of *Cryptosporidium* spp. positive cases from the participating laboratories was also unexpectedly high. Preliminary results show that the majority of positive samples in 2013 were *C. parvum*, more than 70%, while the *C. hominis* IbA10G2 genotype was only found in less than 10% of cases. GP60 PCR and sequencing is a time consuming and expensive method for subtyping *Cryptosporidium* spp. In times of an outbreak, rapid results are helpful to get an indication as to whether an outbreak occurring or a (seasonal) increase. To reduce time and costs, we developed a multiplex PCR to detect *Cryptosporidium* spp. and subtype *C. parvum* and *C. hominis* in one test. In doing so, we were able to confirm the results of the *Cryptosporidium* spp. typing in 2012 and we implemented these methods for the study in 2013.

# Poster Presentations

P75

## First molecular characterisation of *Giardia duodenalis* in Algeria

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*Giardia duodenalis* is an enteric protozoan parasite, which infects human and a wide range of vertebrate hosts. In Algeria, this parasite is common and well known, but no genotyping is conducted so far. The goal of this study was to investigate genotypes of *G. duodenalis* from children fecal samples in Reghaia, east of Algiers Algeria, by Nested PCR and sequencing the triose phosphate isomerase (TPI) gene, after DNA extraction using the Fast DNA Spin Kit Fast For Soil, from 68 children fecal samples who were positive for *G. duodenalis* by microscopy. The positive results were confirmed for all the samples by nested PCR. The 68 isolates were successfully sequenced and indicated the dominance of Assemblage B (59%) and Assemblage A (33%). This study is the first to indicate the molecular characterization of *G. duodenalis* in east of Algiers. The high frequency found and the genotypes diversity found suggest various sources of contamination of *Giardia duodenalis* to human.

**Keywords:** *Giardia duodenalis*, Triose phosphate isomerase (TPI), nested PCR, sequencing, Algeria

# Poster Presentations

P76

## **Limitations of the 18S rRNA molecule: implications for molecular taxonomy and phylogenetic reconstruction of *Cryptosporidium***

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The 18S rRNA molecule is an important molecular marker that is used for diverse applications, including species identification, diversity assessments and evolutionary reconstructions. Despite the wide uses the 18S rRNA has inherent issues, and its behavior has indicated that it may not be the most suitable gene for reconstructing phylogenies. For example the variable rates of substitution in different gene regions and rapid divergence of some groups results in polytomies that complicate phylogenetic reconstructions.

For *Cryptosporidium* the 18S rRNA molecule is commonly used for species identification and differentiation, and for inferring evolutionary relationships within the genus. Further, the multi-copy nature of the 18S rRNA makes it a useful target for *Cryptosporidium* screening. Given the issues of this 18S rRNA molecule for other taxa it is important to scrutinise potential issues relative to *Cryptosporidium*. In this presentation the association between *Cryptosporidium* and its marsupial hosts will be used to demonstrate that the inherent problems of the 18S rRNA molecule present issues for its application to *Cryptosporidium* biology. Complications in applications as a screening tool arise as a result of inability of confirmatory loci to amplify 18S rRNA positives, and recent divergence and sequence similarity of some species lead to highly unsupported phylogenetic reconstructions. The importance of understanding these limitations and considering other loci will also be discussed.

# Poster Presentations

## P77 Cell motility in sporozoites of *Cryptosporidium muris*

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Using combined microscopic, biochemical and molecular approaches, this study focuses on cytoskeletal elements that are expected to participate in cell motility of sporozoites of gastric parasite *Cryptosporidium muris*. Although the motility of cryptosporidian sporozoites is considered as the main mechanism facilitating the host cell invasion, our observations show that motility of *C. muris* sporozoites is very limited and featureless, and differs from other apicomplexan zoites. As cryptosporidian sporozoites possess a single rhoptry, they have only one attempt for successful attachment to the host cell. Within the host organism, released sporozoites of *C. muris* rapidly penetrate deeply into the bottom of the pits of the gastric glands to avoid the adverse conditions in the host stomach. In cell cultures with various media, including those enriched by BSA and vitamins, the activity of freshly released sporozoites decreases very rapidly and after several minutes, sporozoites do not show any signs of vitality. The apical region of invasive sporozoites is obviously prolonged and their three-layered pellicle is smooth lacking any grooves or folds. Using immunofluorescence we were able to obtain myosin labelling, which is considered an essential part of apicomplexan motility motor. Labelling of actin with a specific antibody recognizing the actin in *Toxoplasma gondii* and *Plasmodium falciparum* was not successful despite multiple repetitions of staining procedure of *C. muris* sporozoites. However, immunoblotting assays of sporozoites soluble proteins indicated the presence of actin (42 kDa) in relatively low concentrations. In addition, we were able to amplify by PCR and sequence the *C. muris* actin gene from genomic DNA. Using the both immunofluorescence and immunoblotting, we were able to detect  $\alpha$ -tubulin (50 kDa), which represents an elemental component of subpellicular microtubules. Therefore, the currently obtained data support the presence, in *C. muris*, of basic mechanism of apicomplexan motility that is expected to be based on the orientation of the actomyosin motor by subpellicular microtubules.

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# Poster Presentations

**P78      Detection of encystation- and cyst-specific glycoproteins in *Giardia duodenalis* cultures with differential ability to encyst using a novel lectin ligand**

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Encystation is an adaptive response of *Giardia duodenalis* to adverse conditions triggering the biosynthesis of a protective cyst wall made up of proteins including cyst wall proteins 1-3 (CWP1-3) and a major N-acetylgalactosamine (GalNac) homopolymer. We have reported that the assembly of this structure involves the sequential exposure of polypeptide and carbohydrate-rich moieties on the surface of encysting *Giardia* and when encystation is completed cysts become water-resistant. In this work we initially used radio labeling techniques, anti-CWP1 antibodies (mAb5-3C) and lectin probes to detect surface components expressed by encysting cells [18-90 hrs. post-encystation induction, (p.e.i)] - and cysts from two clones derived from WB strain (namely WBC5 and WBC7) which were previously shown to be efficient and deficient to form cysts in vivo using the Mongolian gerbil model respectively. Although the 125I-surface labeling patterns were different in trophozoites from the two clones, the patterns of 125I-labeled purified cysts from these two clones after 42 hrs. p.e.i. were similar, however the ability to form cysts in vitro was significantly reduced in the WBC7 clone. This difference correlated with the expression of CWP1 at 18 and 42 hrs. p.e.i. in WBC5 and WBC7 cultures respectively. Likewise, using the lectin Concanavalin A (ConA) up to 13 glycoproteins with MW in the 58-170 kDa range were detected earlier in encysting WBC5 trophozoites and in cysts from the two cultures. ConA reactivity was blocked by pre-treatment with N-acetylglucosamine (GluNac) and α-D-mannose. Fluorescence and confocal microscopy analyses showed the presence of Con-A-positive material on the surface of encysting cells and cysts from the two cultures that colocalized with anti-CWP-1 staining. In these assays cysts from WBC7 clone showed an abnormal pattern of fluorescent staining with these two ligands. All together these data show that the WBC7 clone is deficient both in vivo and in vitro cyst formation, particularly in cyst wall assembly and that ConA could be used as a novel encystation and cyst-specific marker in *Giardia* differentiation.



# Poster Presentations

P79

## Dynamics of *Giardia intestinalis* mitosomes

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Typical aerobic mitochondria are ATP producing, double membrane-bound, metabolically very active organelles. They are also very dynamic in structure, undergoing constant cycles of fusion and fission events. By contrast, mitosomes of *Giardia intestinalis* are the simplest known mitochondria among all eukaryotes. *Giardia* mitosomes do not produce any ATP and their only function is the assembly of iron-sulfur (FeS) clusters. So far almost nothing is known about the dynamics of the mitosomes during the life cycle of *Giardia* parasites and under iron-dependent metabolic stress. Using Halo Tag technology, we show that *Giardia* mitosomes are steady organelles, which do not fuse or divide during interphase. We attempt to identify the moment of organelle division and segregation during mitosis as well as the compensation for the reduced organelle number after cytokinesis. Compared to tiny mitosomes the endoplasmic reticulum is the most dominant membrane-bound structure of giardia. The absence of the ERMES complex, which is responsible for the interactions between mitochondria and the ER suggests the presence of an unique mode of interaction between these organelles. We are using in vivo enzymatic tagging by bacterial biotin ligase anchored to the outer mitochondrial membrane to find if mitosomes come into contact with other compartments such the ER and the basal bodies. Iron is an indispensable factor for the cell growth and also the pathogenesis. Iron-restricted conditions often lead to massive morphological and metabolic changes in classical mitochondria and hydrogenosomes including the remodeling of the organellar proteome. Therefore, we grew *Giardia* cells in iron-rich and iron-deficient media and followed the impact of the iron stress on the activity of FeS cluster containing enzymes, the levels of FeS cluster biosynthetic machinery and the morphology of the mitosomes. Surprisingly, while the activity of the FeS cluster containing enzymes dropped rapidly neither the levels of ISC proteins nor the number of the mitosomes changed. This suggests that, when compared to mitochondria and hydrogenosomes, mitosomes lost their capacity to respond to iron deficiency, which eventually leads to the cell death.

# Poster Presentations

P80

## Functional complementation approach to study protein degradation in *Giardia lamblia*

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*Giardia lamblia* switches between two morphological forms, trophozoite and cyst, and protein turnover is crucial for this transition. The eukaryotic proteasome is responsible for cytosolic and nuclear protein degradation. As little is known about the proteasome of *G. lamblia*, we have directed our efforts towards understanding one of the proteasomal proteins that is an orthologue of Rpn10/S5a. Rpn10 is a component of the proteasomal lid subunit and has been studied in many well-studied eukaryotes. It functions as a receptor for binding to ubiquitin tags of substrate proteins destined for proteasomal degradation. As *G. lamblia* is refractory to genetic manipulations, we have adopted functional complementation to functionally characterize the *Giardia* protein in the genetically facile yeast *Saccharomyces cerevisiae*. Our studies show that the Rpn10 orthologue of *G. lamblia* is able to bind to ubiquitin in vitro and can functionally complement the phenotype resulting from deletion of the yeast orthologue. However, a domain of the yeast protein that is crucial for regulating ubiquitin binding is truncated in the *G. lamblia* orthologue. We have also studied the cellular localization of this protein. Our studies raise the possibility that architecture and regulation of the proteasome of *G. lamblia* is likely to be different from that present in eukaryotes studied thus far.

# Poster Presentations

P81

## ***Giardia* Epsin: A Novel Membrane Attachment Factor in *Giardia*?**

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*Giardia* trophozoites attach to the epithelium of the host small intestine via a large concave suction disk-like structure composed of a spiral microtubule array covered by the ventral plasma membrane. Despite the striking morphology of this so-called ventral disk, there is not much information about the morphogenesis and composition of this specialized cytoskeleton structure and how the plasma membrane is attached to it.

The fortuitous finding that a giardial Epsin-R homologue (GEpsin) localized specifically to the ventral disk prompted us to investigate a possible role as a linker molecule, connecting the ventral plasma membrane with the disk cytoskeleton. We tested this hypothesis by functional analysis of GEpsin in vivo and in vitro. The classical functions of epsins in eukaryotes are induction of membrane curvature and acting as adaptors for coat protein components in endocytic processes. GEpsin is not involved in membrane trafficking, but we could show that the conserved GEpsin N-terminal ENTH domain interacts very specifically with membrane lipids, suggesting binding to and intercalation into the cytoplasmic layer of the ventral plasma membrane. Although intercalation has not been demonstrated directly, it is consistent with the GEpsin domain structure and with induction of membrane curvature to accommodate the concave shape of the ventral disk. Importantly, we also demonstrate that the unique GEpsin C-terminal domain is necessary and sufficient for targeting the protein to the ventral disk, most likely during reassembly of daughter disks when trophozoites undergo cell division. We are currently implementing strategies to identify direct binding partners in the disk cytoskeleton.

Exaptation of the only Epsin in *Giardia* is yet another striking example how protein domains with fundamental functions (in this case selective binding to membrane lipids and possibly also membrane intercalation) are conserved and reassigned when their original function(s) has become obsolete.

# Poster Presentations

## P82 Preliminary characterization of three *Cryptosporidium parvum* proteins correlated to the Golgi complex.

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Apicomplexans possess unique vesicular organelles dedicated to the invasion of the host cell through the transportation of secreted proteins. Micronemes, dense granules and ropthries constitute an exclusive vesicular system that characterizes these parasites. Differently the Golgi complex is a membranous structure highly conserved in eukaryotes as this organelle is the central connection of vesicular transport that mediates targeting both to intracellular locations and the exterior. As previously described, the Golgi apparatus also plays a crucial role in the protein trafficking of *Toxoplasma gondii* and *Plasmodium falciparum*. Therefore, a better understanding of the apicomplexan Golgi is likely to provide useful insights into the biology and pathogenesis mediated by the distinctive organelles of these parasites. The Golgi apparatus in *Cryptosporidium* species has been described at the ultrastructural level in two species, *Cryptosporidium muris* and *Cryptosporidium parvum*, even though there are no further data about this organelle. To investigate the *C. parvum* Golgi complex, we searched for conserved homologs of Golgi-associated proteins in the predicted proteome of *C. parvum* performing a Blast analysis with different Golgi proteins from Apicomplexa or other unrelated organisms. This analysis identified various *C. parvum* putative proteins likely related to the Golgi functions and three of them, homologs of Sec23, Grasp and Sortilin, were selected for further investigations. The putative genes for these proteins appeared to be constituted of a unique exon without introns. Hence, the ORFs corresponding to these proteins were amplified from the *C. parvum* genome to be cloned in *Escherichia coli* expression vectors. The recombinant Sec23, Grasp and Sortilin were expressed as 6 histidine-tagged proteins, purified and used to immunize mice to obtain specific antisera. The native proteins expressed in *C. parvum* were identified by immunoblot assays with the total lysate from sporozoites probed with the antisera for these recombinant proteins. We observed that anti-Sec23 antibodies recognized a band of 70 kDa in sporozoites, whereas anti-Sortilin antibodies bound a unique band of 80 kDa. Remarkably, antibodies for Grasp recognized a triplet of bands of approximately 90 kDa, suggesting the presence of different variants of this protein similarly to the Grasp from *P. falciparum*. We also investigated the expression of the mRNAs of these genes in excysted sporozoites by RTPCR experiments obtaining amplified cDNAs of the three genes. Immunofluorescence experiments using the antisera for these proteins are in course to localize the Golgi complex and to describe the origin of the vesicular network in *C. parvum*.

# Poster Presentations

## P83 PUF proteins in *Giardia intestinalis*

**Vladimíra Najdová<sup>1</sup>** and Pavel Doležal<sup>1</sup>

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The regulation of gene expression during giardia cell- and life-cycle has been poorly studied so far with the exception of variable surface proteins, which constitute the immunoprotective coat of the cell. Thus, we have decided to characterize one important factor of the regulation machinery. The 3' untranslated regions (3'UTRs) of mRNA mediate the stability as well as the localization of the transcripts. PUF proteins (named after PUMILIO protein in *Drosophila melanogaster* and Fem-3 binding factor in *Caenorhabditis elegans*) represent family of 3'UTR-binding proteins, which control the function of the target transcripts by their repression, activation or sequestration. These eukaryotic proteins are evolutionarily conserved from yeast to humans and plants. Each of them contain highly conserved C-terminal domain, which specific binds to 3'UTR of mRNAs. We have identified five hypothetical PUF proteins in *G. intestinalis* genome and expressed them as C-terminal hemagglutinin-tag fusions. All proteins have cytoplasmic localization with the affinity to the endoplasmic reticulum membrane. We further attempt to characterize the set of cognate mRNAs and partner proteins. These findings will not only provide us with the important insight into the regulation of gene expression in *G.intestinalis* but may also give us the insights into the general mechanism of PUF-mRNA interactions and the evolution of this eukaryotic protein family.

# Poster Presentations

## P84 Characterization of Microtubule-binding and Dimerization Activity of *Giardia lamblia* End-binding 1 Protein

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*Giardia lamblia*, with two nuclei and cytoskeletal structures, requires accurate microtubule (MT) distribution for division. End-binding 1 (EB1) protein is a regulator of MT polymerization. Immunofluorescence assay (IFA) of *G. lamblia* expressing haemagglutinin (HA)-tagged *G. lamblia* EB1 (GLEB1) indicates localization in nuclear envelopes and median bodies, and transient presence in mitotic-spindles of dividing cells. Size exclusion chromatography suggests that recombinant GLEB1 (rGLEB1) is present as a dimer formed by its C-terminal domain and disulfide bond. An intermolecular disulfide bond is made between cysteine #13 of the two monomers. MT-binding assays demonstrate that rGLEB1102-238, but not rGLEB11-184, maintains an MT-binding ability comparable with that of rGLEB11-238. A *G. lamblia* aurora kinase (GIAK) was investigated as an enzyme working on GLEB1. The association between rGIAK and rGLEB1 is shown via coimmunoprecipitation using in vitro-synthesized AK and EB1. rGLEB1 is phosphorylated when it reacts with *Giardia* extracts immunoprecipitated with anti-GIAK antibodies. Kinase assays show that a mutant rGLEB1 at serine residue #148 cannot be phosphorylated. These results demonstrate that GLEB1 is involved in the regulation of MT distribution via interaction with GIAK.

# Poster Presentations

P85      **A family of heterogeneous telomerically-encoded *Cryptosporidium* glycoproteins (HTEGs) displaying host specific sequence variation.**

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Genetic variability is limited between the two major human-infective species of Cryptosporidiosis, *Cryptosporidium parvum* and *Cryptosporidium hominis*, a fact which makes it difficult to identify the genetic basis of differential host specificity and virulence. We have previously characterized two proteins, encoded telomerically which display characteristics commonly observed in genes involved in host specificity and virulence. Here we describe the further characterization of an extended HTEG group. Four new members of the HTEG family were identified in silico, including a telomeric gene at the end of chromosome 6 in *C. parvum*, its ortholog in *C. hominis*, a telomeric gene at the end of chromosome 5 of *C. hominis* and its ortholog, which appears to be internal, on chromosome 5 of *C. parvum*. Initial screening across a wide range of *Cryptosporidium* species with an internal portion of the gene on chromosome 6 of *C. parvum* amplified in most *Cryptosporidium* strains and species but showed considerable divergence between zoonotic forms of *C. parvum* and *C. baileyi* compared to anthroponotic *C. parvum* and *C. hominis*. Protein divergence was inferred by calculating the ratio of non-synonymous to synonymous SNPs. Analysis of the divergence between zoonotic and anthroponotic reference strains of *Cryptosporidium* provided a preliminary indication that these genes may be involved in adaptation to the human host. Inclusion of further zoonotic and anthroponotic strains will test this link. The development of these genes for improved species specific assays in diagnosis and risk assessment is described.

# Poster Presentations

**P86      Expression and purification of *Cryptosporidium hominis* recombinant glycoprotein GP15 in *Escherichia coli***

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*Cryptosporidium* spp. are protozoa which infect a wide variety of vertebrates. *Cryptosporidium hominis* is a species adapted to the human host. Among the various genetic markers that are used for specific identification of *Cryptosporidium*, the locus most commonly used for molecular epidemiology studies is the gene that encodes the glycoprotein also known as GP15/40; the GP15 portion of this locus contains several regions with high mutation rates and is associated with the adhesion of the parasite to the host cell. In this study, the expression of GP15 protein of *C. hominis* in *Escherichia coli* has been accomplished by the synthesis (GenScript, USA) of the full-length nucleotide sequence of *C. hominis* GP15 gene followed by cloning and expression with the Champion™ pET SUMO Protein Expression System kit (Life Technologies). Temperature, time and inducer concentration assays were also performed and analyzed by SDS-PAGE gel. After the expression, the protein purification was performed by affinity chromatography using Hi-Trap HP column for ÄKTATM Pure System. The resulting recombinant GP15 glycoprotein can be useful for developing vaccines, new diagnostics methods and treatment for cryptosporidiosis.



# Poster Presentations

**P87 Expression and purification of *Cryptosporidium parvum* recombinant oocyst wall protein 6 (COWP6) *Escherichia coli***

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*Cryptosporidium* spp. are protozoa which infect a wide variety of vertebrates. *Cryptosporidium parvum* has many animal hosts, including humans, and is a major etiological agent of diarrhea in ruminants. Among the various genetic markers that are used for specific identification of *Cryptosporidium*, the *Cryptosporidium* oocysts wall proteins (COWP) are a group of proteins located on the surface of the oocyst wall, are involved in its structure and resistance of oocysts in environmental conditions. COWP proteins are potential targets for cloning and heterologous expression in *E. coli*, for the production of recombinant proteins useful for the development of diagnostic methods like direct immunofluorescence assay (DIA), and also for isolation and purification of oocysts through immunological techniques. In this study, the protein expression in *E. coli* has been made targeting COWP6 of *Cryptosporidium parvum*, that is considered the most important zoonotic species. The full-length nucleotide sequence of *C. parvum* COWP6 gene was amplified by PCR with the addition of a serine at the 5' end of the forward primer and using a high fidelity Taq DNA Polymerase (Thermo scientific, USA). The gene sequence was cloned and expressed with the Champion™ pET SUMO Protein Expression System kit (Life Technologies). Temperature, time and inducer concentration assays were also performed and analyzed by SDS-PAGE gel. After the expression, the protein purification was performed by affinity chromatography using Hi-Trap HP column for ÄKTATM Pure System. The resulting *C. parvum* COWP6 recombinant protein will be used for production of anti-COWP6 chicken IgY aiming the development of diagnostic assays.

# Poster Presentations

## P88 Gene duplication among *Giardia duodenalis* assemblages

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Giardiasis is one of the most prevalent protozoan diseases worldwide which is caused by *Giardia duodenalis*, a representative member of Diplomonads and an early branching group in the evolutionary history of eukaryotes. This parasite is classified in eight genetic assemblages, which infect diverse species of mammals. Among these assemblages A and B infect humans. Genome analyses of assemblages A, B and E have been widely performed and the data obtained suggest that gene diversity and abundance varies among assemblages, a trait possibly associated with the evolutionary history of *Giardia*.

Among the molecular mechanisms involved in the generation of new genes, gene duplication was the first to be described, and it occurs in all known lineages of organisms. Based in whole genome analyses, two major events of gene duplication have been described in *G. duodenalis* Assemblage A which are probably associated with the adaptation of this protozoan to a parasitic life style.

In this study we searched for duplicated genes in the sequenced genomes of assemblages A, B and E. Protein sequences of the same genome were compared in an all against all manner using BLASTp with an E-value cutoff of 10<sup>-4</sup>. Results were filtered and classified as full duplicates and partial duplicates. Based on these results similarity networks were constructed using Cytoscape, and duplication patterns were compared among assemblages.

Duplication patterns varied significantly and Assemblage E showed the highest proportion of duplicated genes (41%) followed by Assemblage B (33.9%). In the three assemblages, the NEK 21\_1 kinases seem to be the most frequently duplicated group of genes.

Interestingly, the VSPs category of duplicates was among the most variable category of genes. In this group, Assemblage B does not show a representative for this group of genes. Furthermore Assemblage E has a peculiar topology, in which VSPs are related to a single VSP, probably the common ancestor, but they are not significantly similar to each other, suggesting that their duplication occurred independently and most likely in a sequential manner from the same ancestral gene. In terms of the categories of duplicates little variation was observed among assemblages, however, substantial variation was observed in the proportions and topologies of duplication patterns. These data reveal different gene duplication patterns among the different assemblages.

All together these findings support the separation of *Giardia* assemblages in distinct species considering that gene duplication is a driving force in the evolutionary history of this genus and that this may be related with its parasitic lifestyle.

**P89      Genomic variation within *Giardia intestinalis* Assemblage A isolates: characterization of genes under positive selection**

**Dimitra Peirasmaki**<sup>1</sup>, Johan Ankarklev<sup>1</sup>, Oscar Franzén<sup>2</sup>, Jon Jerlström-Hultqvist<sup>1</sup>, Marianne Lebbad<sup>3</sup>, Jan O Andersson<sup>1</sup>, Björn Andersson<sup>4</sup> and Staffan G Svärd<sup>1</sup>

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*Giardia intestinalis* makes up a species complex of eight different assemblages (A-H), where Assemblage A and B infect humans. We have performed whole genome sequencing of two sub-Assemblage AII isolates, recently axenized from symptomatic patients, to study the genetic diversity within A and to identify genes under positive selection. Several biological differences between the Assemblage A isolates were identified, including a difference in growth medium preference. The two AII isolates were of different sub-Assemblage types (AII-1 (AS98) and AII-2 (AS175)) and showed size differences in the smallest chromosomes. The amount of genetic diversity was characterized in relation to the genome of an Assemblage AI isolate (WB). Our analyses indicate that the divergence between AI and AII is approximately 1%, represented by ~100,000 single nucleotide polymorphisms (SNP). Moreover, SNPs are homogeneously distributed over the chromosomes with an enrichment in regions containing surface antigens. The level of allelic sequence heterozygosity (ASH) in the two AII isolates were found to be 0.25-0.35%, which is 25-30fold higher than in the WB isolate. 35 protein-encoding genes, not found in the WB genome, were identified in the two AII genomes. The large gene families of variant-specific surface proteins (VSPs) and high cysteine membrane proteins (HCMPs) showed isolate-specific divergences of the gene repertoires. Certain genes, often in small gene families with 2 to 8 members, showed high sequence diversity between the Assemblage A isolates, suggesting that they are under positive selection and they could have important roles in host-parasite interactions. One of the families, Bactericidal/Permeability Increasing (BPI) protein, with eight members was characterized further and the proteins localize to ER with potential lipid-binding activity. Our results show that there is a significant genomic variation in Assemblage A isolates, in terms of chromosome size, gene content, surface protein repertoire and gene polymorphisms.

# Poster Presentations

## P90 *Giardia lamblia*'s Protein-Protein Interaction Network

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*Giardia* is a genus of anaerobic flagellated protozoan parasites of the phylum Diplomonada, in the supergroup "Excavata", which parasitizes the intestine of vertebrates, being one of the most common causes of human diarrheal disease worldwide. During its life cycle, *Giardia* alternates between the disease-causing trophozoite and the hardly dormant cyst that contaminates water or food. *Giardia* comprises a group of true eukaryotic organisms, since they have two nuclei, a vastly extended endomembranous system, and a complex cytoskeleton. However, this group also possesses several prokaryotic features, including reduced metabolic pathways and the absence of organelles typical of more derived eukaryotes, such as mitochondrias, peroxisomes, and a Golgi apparatus. Protein interaction network (PIN) description plays an essential role in the understanding of any organism's cellular biology. Due to the lack of experimental data regarding protein interactions in *Giardia* species, we have taken a computational approach to predict the PINs of three *Giardia lamblia* isolates (A, B, and E), also known as assemblages WB, GS and P15. In this work, the Interlog Mapping method was used. Specifically, on this approach, it assumes that if two proteins have a great sequence similarity against two proteins from a public data base, and these latter ones interact, then the former ones interact as well. Using this methodology, we predicted the PINs for isolates A, B, and E of *Giardia lamblia*. These networks comprise more than 1,500 nodes (25% of the genome), and about 20,000 interactions each. Topological and functional analysis showed that, even though *Giardia* lack structural and molecular complexity, the networks are organized in a modular manner, suggesting that the reduced number of molecular components present in this organisms are sufficient to support biological function. Moreover, using degree and centrality measures, we were able to select putative essential proteins for cell cycle completion and survival of parasite. We have constructed the first PIN of *Giardia lamblia* by using a computational approach. The topological and functional analysis of the resulting PIN enabled us to describe aspects of the biology of these organisms from a previously unexplored point of view.

# Poster Presentations

P91      **Homology modeling of *Giardia intestinalis* arginine deiminase: Insights into its structure and inhibition**

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The arginine deiminase (ADI) of *Giardia intestinalis* is an important arginine metabolic enzyme involved in the energy production and defense in this protozoan. ADI is an attractive target for drug design against *G. intestinalis* because it is absent in the human host. Unfortunately, at present time the crystallographic structure of *G. intestinalis* ADI remains unresolved. Because of its relevance, in this work we present a study that generated a tridimensional theoretical model for the ADI homodimer structure of *G. intestinalis* using a computational technique homology modeling. The sequence alignment, secondary and three-dimensional structure generated shows amino acids conservation at the active site compared with ADI in other organisms. Also, in order to propose possible active-site inhibitors, a set of 3,196 commercial and 19 in-house benzimidazole derivatives were docked into the active site cavity. Molecular dynamics were carried out to evaluate the stability of the dimer enzyme and the ligand-enzyme complexes. The results allowed the identification of molecules with theoretically high affinities for the catalytic site, and thus revealing the most relevant interactions that promote stabilization in it. These results constitute valuable information for the design and optimization of selective inhibitors.

# Poster Presentations

## P92 **CryptoDB and GiardiaDB: Free Online Resources for Genomic, Functional Genomic and Isolate data from *Giardia* and *Cryptosporidium* Species**

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CryptoDB (<http://cryptodb.org>) and GiardiaDB (<http://giardiadb.org>) are components of the eukaryotic pathogen databases (EuPathDB.org). These resources provide free online access to the genomes, functional genomics and isolate data from *Cryptosporidium* sp. and *Giardia* assemblages. Underlying data are searchable via an intuitive graphical user interface that allows the development of complex in silico experiments to support hypothesis driven lab work. Data types include the underlying genomic sequences and annotations, transcript level data (SAGE-tag, EST, microarray and RNA sequence data), protein expression data, population-level (SNP) and isolate data. In addition, genomic analyses provide the ability to search for gene features, subcellular localization, motifs (InterPro and user defined), function (Enzyme commission annotation and GO terms) and evolutionary relationships based on gene orthology.

Highlights from CryptoDB and GiardiaDB:

- Community annotation and curation via user comments (including images, files, PubMed records,etc) can be added to records in CryptoDB and GiardiaDB. Comments become immediately visible and searchable.
- Graphical search system allows building complex searches in a step-wise manner. Strategies can be saved, modified and shared. An example strategy can be viewed by following this link: *Giardia* genes with evidence of expression in the basal body and with evidence of expression by

RNA sequence data: <http://giardiadb.org/giardiadb/im.do?s=3527b7488980e18d>

*Cryptosporidium* isolates identified in Asia from non-human sources, and typed using the GP40/15 locus: <http://cryptodb.org/cryptodb/im.do?s=c180f042cf461b63>

- A genomic colocation tool enables searches based on the relative genomic locations. For example, identifying genes based on their location relative to a DNA motif.
- Column analysis tools are available to generate word cloud graphics and histograms of results. Additional tools will be added in the near future including GO term and pathways enrichment analyses.

# Poster Presentations

## P93 Protein proximity-labeling techniques in diplomonads: progress and challenges

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The diplomonad fish parasite, *Spironucleus salmonicida*, is a diplomonad flagellate and a close relative of *Giardia*. Its genome was recently published and that has made it possible to extensively research this parasite and use it as a model system for other eukaryotes.

Our work has mainly involved development of different protein labeling techniques where we incorporate ascorbate peroxidase (APX) and mutated variants of it (APEX, tdAPX, APEX2) as protein tags to provide localization of a fusion partner. Our aim is to develop protein localization systems using *S. salmonicida* that are transferrable to other diplomonads and eukaryotes. The benefits of using APX instead of another peroxidase such as horse-radish peroxidase is that it is 40% smaller and has been shown to be active in many compartments of the human cell, even the mitochondria. These techniques involve the use of different substrates for the peroxidase, creating precipitates at the location of the tagged proteins allowing the tailoring of the substrate to the experiment at hand. We have already established fusions and labeling conditions for two substrates, Amplex Ultra Red and 3,3'-Diaminobenzidine tetrahydrochloride (DAB). Using Amplex Ultra Red, the peroxidase catalyzes a reaction between the substrate and  $H_2O_2$  which creates a precipitate called resorufine, a red-fluorescent oxidation product, at the location of the tagged protein, which can be viewed in a fluorescence microscope. When using DAB, the peroxidase catalyzes the oxidation of the substrate by  $H_2O_2$ , generating a dark brown precipitate at the tagged protein location, which can be viewed in a bright field microscope. Furthermore, the osmophilic nature of the precipitate means that it is observable also by electron microscopy.

Our current work has involved the development of a protein proximity-labeling method using another APX substrate, biotin-phenol, where we are interested in biotinylating proteins in close proximity to the tagged protein. The biotinylated proteins can be purified using streptavidin beads and identified using mass spectrometry. This could be very beneficial in detecting interacting and unknown proteins. For the same purpose, we have been developing another proximity labeling method using a protein-biotin ligase called BirA\*, where we use the BirA\* with excessive biotin concentration to biotinylate neighboring proteins, which then can be purified using streptavidin beads and analyzed with mass spectrometry.

# Poster Presentations

P94

## **Whole Genome Amplification from clinical samples: Advances in *Cryptosporidium* genotyping from limited numbers of oocysts**

**Jenna L Alexander**<sup>1</sup>, Stephen J Hadfield<sup>2</sup>, Guy Robinson<sup>2</sup>, Kristin Elwin<sup>2</sup>, Rachel M Chalmers<sup>2</sup> and Justin A Pachebat<sup>1</sup>

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The study of *Cryptosporidium* is hampered by an insufficient understanding of species sub-types and diversity. Whole genome reads can give a wealth of information about infectivity, phylogeny, and host specificity, but to date only three species have been genome sequenced and published. Recent advances in next generation sequencing (NGS), and the use of the transposon based Illumina Nextera XT sequencing library preparation kit, has allowed us to characterise *Cryptosporidium* genomic sequences directly from clinical samples from which over a nanogram of DNA can be extracted (See Hadfield et al, Pachebat et al abstracts). One limiting factor in the generation of sequence data directly from isolates is the small number of oocysts that can be recovered from clinical samples, often resulting in the extraction of subnanogram quantities of genomic DNA. Here we discuss the application of single molecule genomic DNA extraction methods to improve the recovery of genomic DNA from oocysts, and the efficiency of commercial available single cell whole genome amplification (WGA) kits to amplify a range of genomic DNA concentrations isolated from *Cryptosporidium parvum* oocysts. We also discuss NGS sequence characterisation of the WGA amplified DNA to determine the efficacy of the various WGA methods, and the application of this method to clinical isolates stored at the Cryptosporidium Reference Unit.



# Poster Presentations

## P95 Characterisation of a *Cryptosporidium viatorum* human isolate by next generation sequencing

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*Cryptosporidium viatorum* is a novel *Cryptosporidium* genotype first isolated by Elwin et al [1] from patients with gastro-intestinal symptoms returning from the Indian sub-continent<sup>1</sup>. It was reported that patients showed slightly different symptoms from those infected with *Cryptosporidium hominis* and *Cryptosporidium parvum* with less reported vomiting but longer duration of gastro-intestinal symptoms [1]. Using a process developed to isolate, extract and sequence oocyst DNA directly from clinical samples, we have performed next generation sequencing of the *C. viatorum* genomic DNA from a clinical isolate. Here we discuss the assembly and in-depth sequence analysis of the *C. viatorum* genome.

### Reference:

[1] Elwin, K et al 2012. *Cryptosporidium viatorum* n. sp. (Apicomplexa: Cryptosporidiidae) among travellers returning to Great Britain from the Indian subcontinent, 2007-2011. Int. J. Parasitology 42:675-82

P96

## **Transcriptional Profiling of Differentiated CaCo-2 Intestinal Epithelial Cells Response to *Giardia Intestinalis* during Early Onset of in vitro Interactions: Insights into the Pathways of Cytokine Production and Regulation**

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*Giardia Intestinalis* is a protozoan parasite that causes diarrhea in humans. The disease has a global distribution with a major impact on children of the developing world. Although great advancements have been achieved in understanding *Giardia* biology, the exact disease mechanisms are not completely understood. It is also unknown how *Giardia* evades immune responses and inhibits intestinal inflammation including interleukin-8 (IL-8) production. Therefore, this study aimed to underpin transcriptional changes in parasitised cells during early hours of interactions with *Giardia* (1.5h, 3h and 4.5h) with a major focus on genes involved in immune response activation/inhibition. We used RNA Sequencing to analyse the transcriptome of differentiated CaCo-2 intestinal epithelial cells in response to the WB (Assemblage AI) and AS175 (Assemblage AII) isolates in vitro. Transcriptional changes in CaCo-2 cells, in response to each isolate, were highly correlated ( $r=0.93$ ) at 1.5h of interaction but not at 3h and 4.5h. The transcription of genes involved in NF $\kappa$ B signaling (inhibitors of NF $\kappa$ B, mitogen activated protein kinase (MAPK) and apoptosis regulators) was induced, concurrent with the release of cytokines/chemokines (IL-8, CCL2, CCL20, CXCL1, CXCL2 and CXCL3) into the interaction medium as confirmed by ELISA; IL-8 was the least induced. Genes associated with the regulation of transcription, apoptosis, cellular proliferation and adhesion were amongst the highest up-regulated genes (>5fold increase). Some interesting genes are those with anti-inflammatory functions (tumor necrosis factor alpha induced protein 3, tnfaip3, zinc finger protein 36 C3H type, zfp36, zinc finger CCCH type containing 12A, zc3h12a, and nuclear factor kappa light polypeptide gene enhancer in B cell inhibitor, nfkb1). Amongst those, zfp36 and zc3h12a, might mediate the regulation of cytokine production via binding the AU rich elements (AREs) in the 3'UTR of many cytokine genes, affecting mRNA stability. At 3h and 4.5h, most of the up-regulated genes with pro-inflammatory functions and those involved in NF $\kappa$ B signaling were down-regulated. The transcriptional response in differentiated CaCo-2 was mainly indicative of the effect *Giardia* has on epithelial cell integrity and cytoskeleton (plau, fbxo16, krt13, mmp3, fbxl2). The results show that *Giardia* induces immune responses in host cells, mediated by NF $\kappa$ B activation and MAPK signaling, immediately following infection peaking at 1.5h but dropping thereafter. A role for AREs binding proteins is suggested in the regulation of cytokine production during giardiasis mainly by affecting mRNA stability. This study has provided more insights into understanding host transcriptional and immune responses to *Giardia*.

# Poster Presentations

P97

## ***Cryptosporidium parvum* IId family: clonal population and dispersal from Western Asia to other geographical regions**

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In this study, 111 *Cryptosporidium parvum* IId isolates from several species of animals in China, Sweden, and Egypt were subtyped by multilocus sequence typing (MLST). One to eleven subtypes were detected at each of the 12 microsatellite, minisatellite, and single nucleotide polymorphism (SNP) loci, forming 25 MLST subtypes. Host-adaptation and significant geographical segregation were both observed in the MLST subtypes. A clonal population structure was seen in *C. parvum* IId isolates from China and Sweden. Three ancestral lineages and the same RPGR sequence were shared by these isolates examined. Therefore, the present genetic observations including the higher nucleotide diversity of *C. parvum* IId GP60 sequences in Western Asia, as well as the unique distribution of IId subtypes (almost exclusively found in Asia, Europe, and Egypt) and in combination with the domestication history of cattle, sheep, and goats, indicated that *C. parvum* IId subtypes were probably dispersed from Western Asia to other geographical regions. More population genetic structure studies involving various *C. parvum* subtype families using high-resolution tools are needed to better elucidate the origin and dissemination of *C. parvum* in the world.

# Poster Presentations

## P98 **Whole genome sequencing without prior parasite culture - an option for broad genetic characterization of human *Giardia* samples**

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**Background:** Culturing of the intestinal protozoan parasite *Giardia lamblia* is difficult, particularly so for Assemblage B. Genotyping of clinical isolates for studies of epidemiology, symptomatology, virulence and drug resistance have therefore been limited to PCR and sequencing of one, or a few, specific genes. Recently genomes of three culturable isolates, representative of Assemblage A1 and A2 and Assemblage B, have been sequenced. The aim of the present study was to evaluate the performance of whole genome sequencing of purified cysts from clinical isolates.

**Methods:** *Giardia* cysts from two clinical isolates, one Assemblage A and one Assemblage B, were purified by sucrose flotation (SF) twice. The Assemblage B isolate was also further purified by immunomagnetic separation (IMS). DNA was extracted using the Qiagen Stool Kit, and sequenced on a 5500 SOLiD System. LifeScope Genomic Analysis Software v.2.5.1 for SOLiD Next-Generation Sequencing and the genomic.resequencing.frag workflow was used for mapping the resulting reads to *Giardia* genome Assemblage All (DH) and B (GS).

**Results:** The DNA extraction yield was low for all isolated clinical samples, but lowest for the Assemblage A sample indicating a low faecal cyst concentration in this sample. There was an inverse relationship between sample purity and total DNA concentration. Mapping against matching reference genomes, revealed a 1x genome coverage of >90% for both clinical samples and both purification methods. The percentage of mapped reads reflected the cyst purity and pre sequencing DNA quantity. The IMS purified sample had the highest depth with 89.5% of the genome covered 10x. For Assemblage B SF and IMS samples coverage was 100% for three genes important for genotyping (tpi, ghd and  $\beta$ -giardin) as well as for five selected genes determining virulence (depth 7x-75x) and seven drug metabolism genes (depth 1x-73x). The x20 coverage of the IMS sample was on average 40 percentage points higher than for the SF sample. In the Assemblage All sample the three genotyping genes had a coverage of 76-90%, 77% coverage and above for four virulence genes (depth 0-17x) and 89% and above for six drug metabolism genes (depth 0-18x).

**Conclusion:** Whole genome sequencing of purified clinical isolates was shown to be possible, and can fully cover important genes used for genotyping as well as a large range of genes of interest for evaluation of virulence and drug resistance.

# Author Index

Aaen, H P51  
Abaidoo, R C P7  
Abdallah, A M P52  
Adak, G K O12  
Adjei, A A P71  
Adjei, D N P71  
Adjou, K O29  
Advani, R O81, O82  
Aebischer, T O48, O55, P54  
Agoulon, A P8  
Aguayo-Ortiz, R P91  
Aguiar, J M P66, P69  
Aguilera, M P47  
Aholu, H P34  
Al Naimi, S O34  
Aldeyarbi, H M O47  
Alexander, C O54  
Alexander, J L P94  
Aliouat, El M O63  
Aliouat-Denis, C-M O23  
Allgardsson, A O81  
Alm, E O53, O81, O82  
Almeida, I C O39  
Alsarakibi, M P29  
Alsmark, C O81, O82  
Amati, M P38  
Amer, S P97  
Amin, M M G P12  
Amundsen, H P15  
Andersson, B P89  
Andersson, J O O78, O80, P89  
Andersson, M P96  
Andre, C O12  
Anduro-Corona, I P55  
Anim-Baidoo, I P71  
Ankarklev, J P53, P89, P96  
Ansell, B R E O76  
Argüello-García, R O26, O36, P32, P36, P78  
Armah, G P71  
Arrighi, R B G O81, O82  
Arroyo-Salgado, B P48  
Astiazarán-García, H P55  
Ástvaldsson, Á O78, O80, P93  
Atencia, R O20  
Auclair, E O29  
Averaimo, D P13  
Awofisayo, A O11  
Axén, C O6, O14, O81, P14, P65, P97  
Ayaz, E P6  
Ayeh-Kumi, P F P71  
Badroo, G P22  
Baig, M F F P44  
Bailo, B O15, P47  
Bakken, R P98  
Bal, M O19  
Banerjee, S P80  
Baroudi, D P75  
Barrera-Valdes, M de la Luz P35  
Barroeta-Echegaray, E O36, O38  
Bartley, P O45  
Bartolini, R P13  
Barutzki, D P30  
Bazán-Tejeda, M L O26, O38  
Bedi, B O44  
Benamrouz, S O23, O63  
Benavides, J P19  
Berezovski, M V O8  
Bermudez-Cruz, R M O36, O38, O46, P63, P88  
Beser, J O6, O49, O53, O81, O82, P65  
Bhat, M A P22  
Björkman, C O14, P97  
Blanchemain, N P23  
Boots, B O7  
Borkataki, S P22  
Bosaeus-Reineck, H P14  
Boukritaoui, A P75  
Bouزيد, M O34, P67, P85  
Boxall, N S P45  
Boysen, P P51  
Brandão, P E P66  
Brattbakk, H-R P98  
Brigagão, C M P62  
Briggs, A D P45  
Brook-Carter, P T O35

# Author Index

- Brown, C A P71  
Brun-Hansen, H P51  
Buelvas Montes, Y P48  
Bunce, M O52  
Buret, A O33  
Burgess, D P43  
Burrells, A O45  
Buslje, C M P90  
Cabrera-Licon, A O36, P63  
Cacciò, S M O50, P73  
Cameron, S O86, P95  
Camuset, P P72  
Cande, W Z O64  
Canett-Romero, R P55  
Canton, G O45  
Cardona, G A O15  
Carmena, D O15, P47  
Casaert, S P39  
Castillo, R P35, P91  
Certad, G O23, O63  
Chalmers, R M O11, O12, O25, O34, O54,  
O58, O59, O85, O86, O88, P37, P45, P50, P67,  
P85, P94, P95  
Chartier, C O13  
Cheesbrough, J O61  
Chen, Y P26  
Cheun, H-I P42  
Chihai, O O29  
Cho, S-H P42  
Choudhary, S P46  
Chualáin, C N O7  
Claerebout, E O42, P39  
Clark, M P16, P17  
Clime, L P5  
Condlova, S P16  
Cook, N P11  
Corneau, N P5  
Cosford, P O12  
Couture, M O67  
Cozma, V P28  
Cranfield, M P18  
Creusy, C O23  
Crisan, A O79  
Cruz-Soto, M O26  
Dabboussi, F O63  
Dagley, M J O76  
Dakic, Z P41  
Dalsgaard, A O9, P7, P12  
Das, S O39  
Davidson, R K P15  
Davies, A P O58, O59, P50  
De Chatterje, A O39  
De Souza, W P61, P62  
Deacon, A O59  
Debenham, J J O20  
Debosscher, K O42  
Dei-Cas, E O23, O63  
Delafosse, A O13  
Delaire, B O23  
Denno, D M O60  
Derouin, F O30  
Di Cesare, A P13  
DibaoDina, A O31  
Dixon, B R O8, O19, P5  
Doležal, P O70, P79, P83  
Dong, H P25, P26, P97  
Donkor, E S P71  
Dreesen, L O42  
Duarte, T T O39  
Dubourg, A O34  
Dumoulin, M O13  
Dupouy-Camet, J O23  
Dupuy, M-C O13  
Durband, C O61  
Dutoit, E P49  
Ebnetter, J A P81  
Eckmann, L O78, P58  
Efstratiou, A P2  
Efstratiou, M A P2  
Egervärn, M O6  
Ehsan, M A P39  
Eide, G E O62  
Einarsson, E O78, O80, O83  
El Safadi, D O63

# Author Index

- Elkin, B O19  
Elwin, K O25, O34, O85, O86, O88, P37, P85, P94, P95  
Emery, S J O27, O75  
Emmanuel, A O29  
Enemark, H L O9, P7, P12  
Espinosa-Matías, S O36  
Eteti, C P96  
Evans, H O25  
Farber, J P5  
Faso, C O72, O77  
Favennec, L O30, P8, P24, P49, P68, P72  
Feng, C P26  
Feng, Y O51, P97  
Ferella, M P53  
Fina, L O25  
Florent, I P77  
Flores-Benítez, D O36  
Flores-López, V P88  
Foitova, I P20  
Follet, J O23, O31  
Fonesca-Liñan, R O36, O38, P63  
Frace, M O51  
Frank, J P14  
Franzén, O P89  
French cryptosporidium network O30, P49  
French, N O10  
Fuentes, I O15, P47  
Galal, L A P52  
Ganai, A P22  
Ganguly, S P80  
Gantois, N O23  
Ganz, K R P5  
Garcia, J L O45  
Gargala, G O30, P8, P24, P49, P68, P72  
Garibay-Escobar, A P55  
Gati, G P3  
Geary, T O28  
Gee, S O61  
Geldhof, P O42  
Geszy, K O19  
Ghosh, A P80  
Gill, J H O27  
Glavas, S O81, O82  
Godara, R P22  
Gofton, A O52  
Gómez Morales, M A P38  
Gómez-Mondragón, M O36  
González-Mariscal, L O36  
Gorton, R O12  
Gosten-Heinrich, P O55, P54  
Gourgechon, S O62  
Goyal, K P46  
Grabherr, M P53, P96  
Grasset-Chevillot, A O29  
Griffin, S P37  
Grigg, M E O87  
Grinberg, A O56  
Gulliksen, S M P51  
Guo, Y O51  
Guy, R O85  
Guyot, K O23, P49, O63  
Gvozdenovic, E P41  
Gyan, B A P71  
Hadfield, S J O54, O86, P94, P95  
Hafez, H M O21  
Hagström, Å P34  
Hahn, J O55  
Hajkova, I P16  
Hamouma-Ghelboun, O O23  
Hamze, M O63  
Hanevik, K O43, O62, P58, P98  
Hansen, A O6, O49, P9  
Haque, R P60  
Harb, O S P92  
Haynes, P A O75  
He, X P25  
Hegarty, M O86, P95  
Hehl, A B O66, O72, O77, P81  
Helmy, Y A O21, O48  
Hernández, J M P35  
Hernández-Campos, A P35, P91  
Hlaskova, L P27  
Ho, J O79  
Hoepfner, M P96  
Hofmannova, L P27

# Author Index

- Holden, N M O7  
Holmberg, M O6  
Horlock-Roberts, K O65  
Hotchkiss, E O17, O45, O54, P64  
Hrdý, I P79  
Hsiao, W O79  
Hu, W P29  
Hua, J P26  
Hubert, B P8  
Hui, R O74  
Hunter, P R O34, P67, P85  
Häggström, P P14  
Höppner, M P53  
Ignatius, R O55, P54  
Ilgová, J P59  
Indic, N P41  
Iñigo-Figueroa, G P55  
Innes, E A O16, O17, O45, P64  
Insulander, M P65  
Iorio, R P13  
Iqbal, A O8  
Isaac-Renton, J O79  
Isaksson, M P34  
Islamovic, S P73  
Jenkins, E J O19  
Jarlström-Hultqvist, J O78, O80, P89, P93  
Jevtovic, Đ P41  
Jex, A R O76  
Jian, F P25, P26, P97  
Johansen, G P51  
John-Stewart, G O60  
Jones, S O25  
Jonsson, N O16, O17, O45  
Joshi, H P46  
Jovanovic, S P41  
Juremalm, M P34  
Kabir, M P99  
Kafatos, G O12  
Kalousova, B P19  
Kapel, N O30, P49  
Karanis, P O47, P1, P2, P4  
Katoch, M P22  
Katoch, R P22  
Katzner, F O16, O17, O45, P64  
Kazemi, B P1  
Keselman, A O41  
Kestranova, M O18  
Keuh, S O22  
Kevin, T M O34  
Khalil, I A A-M O3  
Khelef, D P75  
Kim, H P P57  
Kim, J P57, P84  
Kissinger, J C O73, P92  
Klotz, C O48, O55, P54  
Kluge, A P54  
Knipe, K O51  
Koinari, M O22  
Kolisko, M O69  
Koloren, Z P6  
Konarzewski, M P31  
Korac, M P41  
Korhonen, P K O76  
Korpe, P P60  
Kortbeek, T M O24, O50, P74  
Kotkova, M O18  
Kotloff, K O1  
Kroes, M P74  
Krücken, J O21, O48  
Ku, B P42  
Kutz, S P15  
Kvac, M O18, P16, P17, P18, P19, P20, P27  
Kvetonova, D O18, P18, P19, P20, P27  
Lacey, E O27, O75  
Laine-Prade, V O29  
Lamden, K O61  
Langeland, N O43, O62, P58, P98  
Larsson, P O81  
Laryea, C E P71  
Le Goff, L P8, P24, P68, P72  
Lebbad, M O49, O53, P65, P89  
Lee, H Y P57



# Author Index

Lee, S-E P42  
Lee, W-J P42  
León-Avila, G P35  
Leturnier, G P31  
Li, E O41  
Li, G P29, P97  
Lie, N O P15  
Lilja, M O57  
Lindberg, T O6  
Lindh, J O57  
Lindström, L O14  
Liu, A P97  
Liu, Y P29  
Llinas, M O71  
Lochlainn, L P74  
Loisy-Hamon, F P31  
Lu, M P60  
Lubberts, E O42  
Ludington, J G P56  
Ludovisi, A P38  
Luo, Q P29  
Luyckx, K P15  
Lv, C P97  
Lymbery, A O22  
Löfdahl, M O49  
Ma, D-W P42  
Ma, J P60  
Ma'ayeh, S Y O35, O84, P96  
Mahmoudi, M R P1  
Maloney, J O41  
Mank, T O24  
Marden, J-P O29  
Marek, S P54  
Marín, C O15  
Marková, K O68  
Martin, A P51  
Martincová, E O69, O70, P79  
Martínez-Espinoza, R O26, P36  
Martínez-Miguel, R M O46  
Martinez-Reyes, D A O46  
Marvin, J O41  
Mason, B W O58, P50  
Matadamas-Martínez, F E P35  
Mateo, María P47  
Mateo, Marta P47  
Mayhead, P O59  
Mazourová, V P77  
McCarthy, N D P45  
McConville, M J O76  
McEvoy, J O18, P16, P17, P27  
McKerr, C O12  
McNair, N N O44  
Mead, J R O44  
Meireles, M V P86, P87  
Melicherová, J P59, P77  
Mendez, T L O39  
Méndez-Estrada, R O P55  
Menotti, J O30  
Meráz-Cruz, N O36  
Midlej, V P61  
Midtgaard, F O20  
Minetti, C O61  
Mirzaqavami, M P33  
Mitrovic, N P41  
Modry, D P18, P19  
Molin, E P96  
Montoya, A P47  
Moriarty, E M O10  
Morine, M O22  
Morrogh-Bernard, H P20  
Morton, S O12  
Muharemagic, D O8  
Mutayoba, B M O20  
Mynarova, A P18, P19, P20  
Mørch, K O62  
Nader, J L O34, P85  
Najdrová, V P83  
Nakamura, A A P86, P87  
Nash, J E O85  
Nash, T E O87  
Naulikha, J M O60  
Ndao, M O28  
Nemejc, K O18  
Ng-Hublin, J O52

# Author Index

- Nguyen, C P20  
Nichols, G L O11, O12  
Nikolic, J P41  
Ning, C P97  
Niu, F P60  
Nocita, E P82  
Nohýnková, E O68  
NoquedaTorres, B P35  
Nordström, A O6  
Nurcahyo, W P20  
Nöckler, K O48  
Oberstaller, J O71  
O'Brien, S J O61  
Odundo, E A O60  
Ofori-Belic, I P41  
Olsen, A O9, P12  
Onchiri, F O O60  
Ongerth, J E O5  
Opsteegh, M O45  
Ortega-Pierres, G O26, O36, O38, P32, P36, P63, P78  
Osman, M O23, O63  
Ottoson, J O6  
Oweson, C O14  
Pachebat, J A O86, P94, P95  
Pacheco-Moreno, B I P55  
Pallant, L P30  
Paoletti, B P13  
Paparini, A O22, O52  
Paraud, C O13, P23  
Parigi, M O45  
Park, S J P57, P84  
Paudyal, S P21  
Pawlik, M O19  
Pavlinac, P B O60  
Pazdzior, R O67  
Paziewska-Harris, A P40  
Paz-Michel, B A P32  
Peirasmaki, D O43, P53, P89  
Penga, L P61  
Petersen, H H O9, P7, P12  
Petersen, T B P7  
Petri, W A Jr O2, P60  
Petrzelkova, K J P18, P19  
Pinel, C O30  
Pirestani, M P33, P70  
Pita, A O10  
Platt, K O61  
Plutzer, J P4  
Pollock, K O12  
Pomajbikova, K P18, P19  
Pors, I O13, P23  
Pottier, M O23  
Power, M P76  
Pozio, E P38, P73  
Prystajecky, N O79  
Puleston, R P37  
Pumpalova, Y O71  
Pyrih, J O69, O70, O71  
Qadeer, A S Q P44  
Qi, M P25, P97  
Quihui-Cota, L P55  
Radbodonirina, M O30  
Radunovic, M P58  
Rafferty, S P O67  
Rahimian, F P70  
Ramírez, J D O87  
Randjelovic, S S P10  
Randle, N O61  
Ras, R O40, O88  
Rascón-Durán, L P55  
Raya-Sandino, A O36  
Razakandrainibe, R P24, P68, P72  
Reaume, C O65  
Renteria Flores, A O28  
Richardson, B A O60  
Richtzenhain, L J P69  
Ring, A-C O6  
Robertson, L J O4, O20, O37, P3, P15, P39, P46, P51  
Robinson, G O86, P94, P95  
Roelfsema, J H O50, P74  
Roellig, D O51  
Roger, A O69

# Author Index

Rortveit, G O62  
Rossa, G A R P66, P69  
Rost, M O18  
Rout, S O72  
Roychowdhury, S O39  
Ryan, U O22, O52  
Saavedra, E P36  
Sadraei, J P33, P70  
Sak, B O18, P16, P18, P19, P20, P27  
Samkova, E O18  
Sammons, S O51  
Sandeman, M R O35  
Sandoval-Cabrera, A P88  
Sane, J P74  
Santos, V A P66, P69  
Sarfati, C O30  
Sarkar, S P80  
Sattar, S A O8  
Saugar, J M O15, P47  
Schallig, H P40  
Schaper, R P30  
Schimmer, B O50, P74  
Schoone, G P40  
Schurer, J O19  
Schwantje, H O19  
Schönning, C P9  
Seeber, F P54  
Seferoglu, O P6  
Seghal, R P46  
Sekikawa, T O32  
Selvarajah, U O59  
Senez, V O31  
Shaw, I O10  
Shrestha, S P P21  
Shutt, K A P19  
Siddique, A P60  
Sikora, P O81  
Silva, R P61  
Silva, S O P66, P69  
Singa, B O O60  
Singer, S M O41  
Sinha, A P80  
Skvarc, M P73  
Skår Saghaug, C O43  
Smith, D O10  
Soares, R M P66, P69  
Šoba, B P73  
Sonzogni-Desautels, K O28  
Sood, S P22  
Spinu, M P3  
Sprong, H O50  
Stadelmann, B P53, P96  
Staels, B O42  
Stenger, B P16, P17  
Stenmark, S O57  
Stensvold, C R P65  
Stevanovic, G P41  
Stiff, R E O58, P50  
Storset, A P51  
Sun, Y P97  
Sung, P O46  
Svenungsson, B O6  
Svärd, S G O36, O43, O78, O80, O83, O84,  
P53, P63, P89, P93, P96  
Sörén, K O6  
Sørnes, S O43  
Tachezy, J O69, O70, O71, P79  
Taku, A P22  
Tang, K O51  
Tang, P O79  
Thomas, D R O25  
Thompson, R P30  
Thomson, J O45  
Thomson, S O16, O17, O45  
Todd, A P19  
Tonanzi, D P38  
Tong, K O22  
Tosini, F P38, P82  
Touz, M C P90  
Traversa, D P13  
Trejo-Soto, P J P91  
Troell, K O6, O14, O81, O82, O83, P14, P34  
Trönnberg, L O6  
Tsui, C O79

# Author Index

Tumová, P O68, P79  
Tyler, K M P67, P85  
Tysnes, K R O37  
Utaaker, K S P46  
Uzlíková, M O68  
Wagner, B O19  
Wagnerova, P O18  
Valdez-Nava, Y P39, P51  
Valigurová, A P59, P77  
Vallee, I O29  
Wallén Norell, A O6  
Walson, J L O60  
Wampfler, P B O77  
Van der Giessen, J W B O50  
Van Pelt, W O50, P74  
Van Santen, D P45  
Wang, H P26, P97  
Wang, M P26  
Wang, R P25, P26, P97  
Wanner, G O68  
Ward, H P37  
Wastling, J M O34, O61  
Weiland, M O83  
VelásquezContreras, C A P55  
Wells, B O16, O45  
Wensaas, K-A O62  
Ventura, J L O36  
Veres, T P5  
Vicente, J-J O62  
Widerström, M O57  
Widmer, G O40, O88  
Villalba Vizcaíno, V P48  
Villena, I P8  
Winiecka-Krusnell, J O49, O81, O82  
Viscogliosi, E O23, O63  
Vitovec, J P27  
Vlandas, A O31  
Voleman, L O70, P79  
Von Samson-Himmelstjerna, G O21, O48  
Woolsey, I O9  
Xia, D O34  
Xiao, L O51, P75, P97  
Xu, F O78, O80  
Yadav, A P22  
Yang, A O67  
Yang, R O52  
Yee, J O65, O67  
Yépez-Mulia, L P35, P91  
Yones, D A P52  
Ytrehus, B P15  
Yuanyuan, X O46  
Zaghlol, K S P52  
Zamani, N P96  
Zamponi, N P90  
Žárský, V O70  
Zemouchi, M P75  
Zentella-Dehesa, A O36  
Zessin, K-H O21, O48  
Zhang, L P25, P26, P97  
Zhang, S P25, P26  
Zhao, G P26  
Zhao, Z P97  
Zheng, G P29  
Zhu, J P26  
Zumthor, J P O66, O72

